National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

NL-339 (r. 88/04)

Canada

THE CONTROL OF BROWN ADIPOSE TISSUE FUNCTION IN MICE WITH GOLDTHIOGLUCOSE-INDUCED OBESITY

by

Judy Eley

A thesis submitted to the School of Graduate Studies of the University of Ottawa in partial fulfulment of the requirements for the degree of Master of Science

Department of Biochemistry
Faculty of Health Sciences
University of Ottawa
Ottawa, Canada

September, 1987

© Judy Eley, Ottawa, Canada, 1988

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-46757-6

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ABSTRACT

The effect of feeding a palatable cafeteria diet or of feeding a restricted amount of chow on brown adipose tissue (BAT) of lean and goldthioglucose (GTG)-obese mice was studied at various times of day and night. The objectives were to discover: (1) whether a previous observation of diet induced growth of BAT of the GTG-obese mouse in the absence of diet-induced thermogenic activation could be explained by a transient stimulation at a time of day not studied (2) whether the increased metabolic efficiency of the food-restricted GTG-obese mouse results from changes in BAT thermogenic activity. In a second study, the time course of cold-induced activation of BAT thermogenesis in lean and GTG-obese mice was assessed. As well, the effects of diet and cold exposure upon the activity of BAT thyroxine 5'-deiodinase (BAT 5'DII) of lean and GTG-obese mice were examined. It was found that a transient activation of BAT thermogenesis, as assessed by changes in BAT mitochondrial GDP binding, does occur in the GTG-obese mouse immediately after the food is presented, but no effect of feeding the diet is seen at other times. Food restriction does not suppress any further the already low level of mitochondrial GDP binding in GTG-obese mice; thus the increased metabolic efficiency of this animal is not explained by alterations in

BAT thermogenic activity. A circadian rhythm in BAT 5'DII activity, differing from that in GDP binding, occurred in both lean and GTG-obese mice. No effect of diet or lesion was observed in this activity. GDP binding was reduced in cold-exposed GTG-obese mice during all measured time points, however this decrease resulted from a lower basal thermogenic activation of the tissue, since cold-induced changes paralleled those seen in lean mice. Consequently the response to cold is concluded to be normal in these animals. Cold exposure caused a large stimulation of BAT 5'DII activity; this increase was initially delayed and attenuated in GTG-obese mice, but within 24 hours approximated that seen in lean animals. In both types of mice 5'DII activity was maximal by 12 hours of cold exposure, and returned to normal levels by 2 weeks. This time course contrasted with the maximum thermogenic capacity (elevated protein content and GDP binding) seen at 2 weeks, again suggesting an independent regulation of these two measures of BAT activation state.

DEDICATION

This thesis is dedicated to my husband, Douglas, who helped me to understand that things are often not what they seem.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. J. Himms-Hagen, for her excellent guidance and endless patience, without which this thesis would not have been possible. I wish also to thank Drs. C. Dulberg and R. Nair for their assistance with statistics. I am also grateful to Douglas Eley and Bob Gricken for their help with the graphics. Finally, I would like to thank the other graduate students in the lab for their advice and friendship, especially Ian who always made time to help.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Energy balance can be simply defined as the balance between energy intake and energy expenditure. For any animal, the size of the body energy stores is dependent on the relationship between these components. If expenditure exceeds intake, an animal enters a state of negative energy balance, and must draw upon body reserves to supply the daily energy requirements. Conversely, if intake exceeds expenditure, a state of positive energy balance is said to exist- the animal will store its excess energy as fat and eventually will become obese. Despite the obvious potential for wide variation, body weight and energy content are known to remain remarkably constant in adult animals of many species, though food availability and energy demands may vary greatly (Le Magnen, 1983). In the past several years, extensive study of this phenomenon has suggested that body energy stores are actively maintained through a complex regulation of both energy intake and energy expenditure (Le Magnen, 1983, Sullivan and Gruen, 1985).

Implicit in our understanding of energy balance regulation is the concept that abnormalities in body energy stores may result from defective control of either or both components of the equation. Thus, in any study of obesity an evaluation of all components of the energy balance is

required. While the intake side of this balance consists of a single variable, that of the food ingested, energy expenditure is the result of several processes. Because the energy metabolized by an animal appears as heat, these can be assessed by measurement of thermogenesis (Lloyd et al, 1978). Thus, the components of thermogenesis, and therefore of energy expenditure, fall into two broad categories. The first is obligatory thermogenesis, which results from the processes which are essential to maintain the integrity of the cell, those which involve the maintenance of a warm-blooded state, and those which are essential for the processing of food (Himms-Hagen, 1983). Obligatory thermogenesis occurs at all times and in most tissues of the living animal. The second component is facultative thermogenesis, which can also be further classified into three main categories: exercise-induced thermogenesis, associated with voluntary activity of the animal, shivering thermogenesis, which occurs at temperatures below thermoneutrality, and non-shivering thermogenesis (NST), which occurs in response to both cold and overeating. In contrast to the obligatory component, the processes involved in facultative thermogenesis are regulated on a moment-to-moment basis, according to prevailing circumstances (Himms-Hagen, 1985a).

The principal site of NST is brown adipose tissue (BAT) (Foster and Frydman, 1978, Rothwell and Stock, 1979,

Girardier, 1983). While exhaustive studies in obese individuals and obese animals have failed to pinpoint a specific defect in the components of obligatory thermogenesis, or in the energy expended for physical activity, the possibility that an abnormality in NST could contribute co the development of obesity has been discussed for many years (Himms-Hagen, 1979, and references therein). Although a role for BAT in NST of rodents, hibernators, and newborn mammals was recognized long ago, because of the small size of this tissue (usually less than 1% of body weight) the remarkable thermogenic capacity of BAT, and thus its potential contribution to energy expenditure was for many years overlooked (Rothwell and Stock, 1985). However, the findings that BAT thermogenesis may under certain conditions account for as much as one third of overall metabolic rate (Foster, 1984), that BAT is the site of diet-induced thermogenesis (DIT) (Rothwell and Stock, 1979) and that BAT thermogenesis is defective in several genetically and experimentally obese animal models (for reviews see Himms-Hagen, 1983, 1984a, 1984b, 1985a) has led to our current concept that thermogenesis in BAT can serve as an energy buffer, and that a defect in this process can contribute to the development of obesity.

Brown adipose is morphologically and functionally distinct from white adipose tissue (WAT). BAT occurs in discrete depots which vary somewhat in location and

appearance, depending upon the species studied. Common sites include the interscapular, subscapular, axillary and intercostal regions, and along the major blood vessels of the abdomen and thorax (Smith and Horwitz, 1969, Himms-Hagen, 1985a, Rothwell and Stock, 1985). Brown adipocytes are multilocular, containing discrete drops of stored triacylglycerol, and are packed with many large mitochondria.

It is a unique proton conductance pathway within BAT mitochondria which bestows upon the tissue its thermogenic capacity (La Noue et al, 1986, Nicholls, 1974, 1976, 1977, Nicholls and Locke, 1984). Activation of this pathway allows reversible uncoupling of respiration from ATP synthesis by permitting dissipation of the proton electrochemical gradient generated by electron transport. Thus, free energy derived from the oxidation of substrate (principally fatty acid) is not stored as ATP, but rather is released as heat. Mitochondrial uncoupling appears to be regulated by the intracellular concentration of fatty acids (Bukowiecki, 1984, Nicholls and Locke, 1984), which therefore act both as substrate and intracellular stimulus for this process. The mechanism is dependent upon a unique protein of molecular weight 32 kDa, located within the inner mitochondrial membrane (La Noue et al, 1986, Nicholls, 1974, 1976, 1977, Nicholls and Locke, 1984). Variously known as nucleotide binding protein, uncoupling protein (UCP), 32,000

M_r protein and thermogenin, (herein referred to as UCP), this protein has been isolated (Lin and Klingenberg, 1982) and an immunoassay for it developed (Cannon et al, 1982, Lean et al, 1983, Ricquier et al, 1983).

NST in BAT is under two distinct forms of regulation; these may be referred to as acute and trophic. The acute response of the BAT adipocyte refers to the extent to which its capacity for thermogenesis is utilized at any one moment. This response has been characterized; for detailed information the reader is referred to several available reviews (Nicholls and Locke, 1984, Nedergaard and Lindberg, 1982, Himms-Hagen, 1983, 1985a). Briefly, acute thermogenic activation of BAT is under the control of noradrenaline (NA) released from sympathetic nerve terminals supplying the tissue. Through an action upon β-adrenergic receptors present on the plasma membrane, NA stimulates the activity of adenylate cyclase, increasing cytosolic cAMP levels and resulting in the activation of several protein kinases. One of these kinases, (as yet unidentified), catalyzes phosphorylation of a hormone sensitive lipase, resulting in liberation of free fatty acids. With the accumulation of free fatty acid the mitochondrion is released from respiratory control. Consequently, a high rate of substrate oxidation becomes possible independent of the need to phosphorylate ADP, and the free energy produced in this process is released as heat.

While the acute thermogenic response of BAT can vary from moment to moment, the maximal capacity of an animal for NST varies with a time course of days, and is dependent upon developmental status, thermal environment, and dietary intake. Thus, an animal responds to its surroundings by developing a maximal thermogenic capacity consistent with its requirements. An adaptive change in the thermogenic capacity of BAT has been termed the trophic response, and occurs after prolonged activation of the sympathetic nerve supply to the tissue (Barnard et al, 1980). The resulting increased thermogenic capacity may arise due to both hyperplasia of the tissue, via division of precursor endothelial cells, and to an increase in cellular protein and mitochondrial mass (hypertrophy) (Bukowiecki, 1984, Bukowiecki and Collet, 1983). Mitochondrial adaptations are also observed; specifically a selective increase in the level of UCP can be measured (Ashwell et al, 1983,1984, Ricquier et al, 1984, Nedergaard et al, 1984, Falcou et al, 1985, Trayhurn et al, 1987). The capacity of BAT for such non-mitochondrial processes as glycolysis and lipogenesis can also be selectively regulated (Cooney and Newsholme, 1984, McCormack, 1982). Thus, an increased thermogenic capacity of the tissue is achieved through increases in the number of brown adipocytes, in the number of mitochondria per cell, in the concentration of UCP per mitochondrion, and finally through increases in the capacity to synthesize and

supply substrate for oxidative metabolism.

The only quantitative method for assessment of BAT thermogenesis is by measurement of oxygen consumption by the various BAT deposits. This may be accomplished through measurement of blood flow using radioactive microspheres and of arterial-venous differences in oxygen tension (Foster and Frydman, 1978, Foster, 1984). However, because this method is technically difficult, a number of in vitro techniques have been developed to give a qualitative index of thermogenic state of BAT. The most commonly used of these measures binding of purine nucleotide, usually guanosine 5'-diphosphate (GDP), to isolated BAT mitochondria (Nicholls, 1976). GDP binding is low in mitochondria from thermogenically quiescent BAT, and increases rapidly when the tissue is acutely stimulated. The UCP has one purine nucleotide binding site per dimer, located on the outer surface of the inner mitochondrial membrane (Nicholls and Locke, 1984) and changes in GDP binding have been interpreted to represent unmasking and remasking of these sites, since they occur rapidly, do not require protein synthesis, and exhibit a diurnal variation (Brooks et al, 1982, Bryant et al, 1983, Desautels and Himms-Hagen, 1979, Rothwell et al, 1983b). Unmasking of GDP binding sites in stimulated BAT mitochondria appears to result from changes in matrix water volume and swelling of mitochondria in the thermogenically active tissue (Nedergaard and Cannon, 1987)

The manner in which changes in BAT activity levels give rise to changes in mitochondrial water volume is, however, presently unknown.

Although some workers (Cannon, Nedergaard and Sundin, 1981) have equated the magnitude of GDP binding with the amount of the UCP, this is not correct. The extent of binding is not directly related to the concentration of this protein, but is a function of both the concentration of binding sites and of the extent to which the sites are exposed and accessible (Himms-Hagen, 1983, 1985; Trayhurn et al, 1987).

Both acute thermogenic activation and the trophic response of BAT are under the control of NA. In many species, activation of sympathetic nerve terminals supplying BAT, as assessed by measurement of noradrenaline turnover (NATR), is promoted by two environmental stimuli. The most effective of these is subjection to cold, that is, to an ambient temperature below thermoneutrality (Young et al, 1982). If such a procedure is carried out for a short period of time, for example less than 24 hours, an animal is considered to be acutely cold exposed, and under normal circumstances will activate cold-induced thermogenesis (CIT) through sympathetic stimulation of its BAT. In this situation an increase in GDP binding to isolated BAT mitochondria can be observed in the rat within 2.5 hours (Trayhurn et al, 1987). This increase is believed to result mainly from the process of unmasking, since changes in GDP

binding can be measured prior to any change in the concentration of UCP. When an animal is subjected to cold for a longer period, such as two weeks, the resulting prolonged sympathetic stimulation produces the trophic changes described above (Bukowiecki and Collet, 1983). Under these conditions, the animal is considered to be cold acclimated, and the increased GDP binding to BAT mitochondria which it displays is presumably due to both an initial unmasking, and to the subsequent trophic increases in total UCP (Trayhurn et al, 1987, Ashwell et al, 1983, Ricquier et al, 1984).

A second stimulus for thermogenic activation of BAT is overeating. While food intake is normally closely regulated in laboratory animals, hyperphagia can often be induced by provision of a "cafeteria diet", consisting of highly palatable (tasty) food items which are varied daily (Rothwell and Stock, 1979, 1983a). When such hyperphagia occurs, sympathetic nervous system activity to BAT is stimulated (Young et al, 1982). If the treatment is prolonged, the tissue will grow and exhibit an increase in UCP content (Falcou et al, 1985, Ashwell et al, 1984, Nedergaard et al, 1984). This increase is, however, much smaller than that attained by cold acclimation, probably due to a weaker and less constant stimulation of the sympathetic nervous system brought on by diet (Himms-Hagen, 1986). Diet-induced thermogenesis (DIT) serves to limit the

development of obesity in the hyperphagic animal, since much of the excess food energy is dissipated as heat. In addition it allows an increased intake of an unbalanced diet, such that an animal may overeat to meet its requirements for specific nutrients while minimizing obesity due to excess energy consumption. DIT may also contribute to the maintenance of euthermia in animals which are exposed to cold.

Central nervous mechanisms which control BAT thermogenesis are those which act via its sympathetic nerve supply. These mechanisms are integrated with regulation of both thermogenesis and energy expenditure (Himms-Hagen, 1985a). An important role for the hypothalamus in this process has been suggested by a variety of different stimulation and lesioning techniques (Perkins et al, 1981, Niijima et al, 1986, Rothwell and Stock, 1982a, Coscina et al, 1985, Hogan et al, 1983, 1985, for example). Activation of BAT thermogenesis by stimulation of the ventromedial region of the hypothalamus (VMH); and the absence of DIT in VMH-lesioned animals have pointed to this area as the primary central regulator of BAT activation in response to diet. CIT, on the other hand, is usually normal in VMH-lesioned animals (Hogan et al, 1982, for example), and appears to be regulated through different hypothalamic areas. These are not yet well defined.

Although many aspects of BAT thermogenic regulation are

similar in the various species studied, in other parameters considerable species difference has been observed. An important example of this statement exists in the contrasting adaptive responses of food-restricted rats and mice. When the food supply of a rat is limited, the BAT of this animal becomes thermogenically quiescent and begins to atrophy. This is reflected in a decreased protein content and reduction in GDP binding (Rothwell and Stock, 1982). Thus, under conditions of limited food supply, the rat conserves food energy by suppressing both BAT thermogenic activation and the thermogenic capacity of the tissue. In the mouse, on the other hand, BAT does not atrophy with food restriction; indeed it is sometimes found to grow (Himms-Hagen, 1985). Nonetheless, GDP binding is reduced in this animal, as is BAT sympathetic activation (Zaror-Behrens and Himms-Hagen, submitted).

In attempting to understand this apparent paradox, one must first consider the ability of the mouse to undergo daily torpor, a metabolic strategy utilized by several species of small size for conservation of energy (Hudson and Scott, 1979, Webb et al, 1980, 1982). Due to the large surface to volume ratio of these animals and to the consequent accelerated rate of heat loss, the energy cost for maintaining euthermia is proportionately much greater than that experienced by larger organisms (McNab, 1983). Thus, when energy supplies are scarce, or when a reduced

ambient temperature increases the rate of heat loss, the mouse may resort to torpor and thus will undergo a temporary departure from euthermia. By decreasing the increment between body surface and ambient temperatures, the rate of heat loss, and therefore of energy expenditure, is reduced.

While the reduction in body temperature (T_b) observed in torpid animals varies with the need to conserve energy, torpor has arbitrarily been defined as the maintenance of a T_b of 31 °C or lower (Hudson and Scott, 1979). This level was chosen in order that drops in body temperature due to random periods of inactivity could be differentiated from true bouts of torpor. These may last for several hours each day, and often occur in the early morning. In contrast to the extremely low temperatures often reached during hibernation, T_b seldom falls below 15 °C during torpor. As well, torpid animals undergo spontaneous daily arousals which vary in frequency and duration according to the energy balance. These arousals allow periods of euthermia during which animals are active and can feed.

During periods of torpor a low T_b is achieved through reduction of thermogenesis in BAT and other organs; thus BAT thermogenic activation, as assessed by the GDP binding technique, is suppressed in torpid mice (Himms-Hagen, 1985b). Measurement of GDP binding during arousal from torpor has suggested that this process may occur through increased BAT thermogenesis, probably mediated by a

transient sympathetic stimulation. These findings may explain the reduced thermogenic activation but normal BAT growth observed in food-restricted mice. Though BAT thermogenesis is suppressed during periods of torpor, a daily stimulation of the tissue during the arousal process may allow maintenance of a normal or increased amount of the tissue. Thus, like the rat, the food-restricted mouse conserves energy during periods of torpor by suppressing thermogenesis in BAT and other organs; unlike the rat, however, it must maintain its BAT in a state that is capable of switching on thermogenesis and precipitating arousal. Interestingly, studies of UCP concentration in mice fasted for 48 hours have yielded conflicting results; in one case a decrease was observed (Trayhurn and Jennings, 1986) while in another no change in the concentration of this protein was detected (Desautels, 1985). UCP levels in chronically food-restricted mice have not been reported.

Although BAT is not a site of thyroid-induced thermogenesis (Himms-Hagen, 1983a, Rothwell and Stock, 1984), thyroid hormone is known to be essential for thermogenic activation of the tissue (Triandafillou et al, 1982). This action results from enhancement of BAT adipocyte responsiveness to catecholamines, through modulation of the response of adenylate cyclase to catecholamine, of the lipolytic response to cAMP, and of the mitochondrial thermogenic response to free fatty acids

(Sundin et al,1984). Given the importance of thyroid hormone in the thermogenic process, it is hardly surprising that BAT can produce its own active thyroid hormone, 3,3',5-triiodothyronine (T_3), from thyroxine (T_4) (Leonard et al, 1983, Silva and Larsen, 1983). Generation of T_3 is accomplished by means of a specific 5'-deiodinase (BAT 5'DII), which differs in its properties from a related enzyme in liver and kidney. Thus, the BAT 5'DII is insensitive to inhibition by propylthiouracil (PTU), is activated by dithiothreitol (DTT) in a sequential mechanism, has a lower Km for T_4 than for 3,3',5'-triiodothyronine (rT_3) and is increased by hypothyroidism (Kaplan, 1980, Silva et al, 1982).

Recent studies have indicated that BAT 5'-DII activity is greatly elevated by acute cold exposure, through action of noradrenaline upon α_1-adrenergic receptors (Silva and Larsen, 1983). During periods of cold-induced sympathetic stimulation, BAT appears to rely upon locally-produced T_3, rather than upon T_3 derived from blood stores (Bianco and Silva, 1987a, 1987b, Bianco and Silva, in press). Indeed, in cold exposed rats and hamsters, T_4 deiodination within BAT may be an important source of T_3 for the general circulation (Kopecky et al, 1986, Fernandez et al, 1987, Silva and Larsen, 1985). It is of note that cold-induced activation of BAT 5'-DII is defective in two models of genetic obesity, the genetically obese (ob/ob) mouse (Kates

and Himms-Hagen, 1985) and the Zucker (fa/fa) rat (Wu et al, 1987). Moreover, both of these animals fail to activate BAT thermogenesis and succumb to hypothermia when acutely cold exposed (Kates and Himms-Hagen, 1985).

Given the mechanism through which BAT 5'-DII activation occurs, one would expect that treatments which enhance sympathetic activity in the tissue must also enhance the activity of this enzyme. At the time the present work was undertaken, the effect of dietary treatment upon BAT 5'-DII activity was not known. It has since been reported that in rats, despite its action to stimulate sympathetic activity, cafeteria feeding either decreases, or is without effect upon BAT 5'-DII activity (Kopecky et al, 1986, Wu et al, 1987). The mechanism by which sympathetic stimulation results in 5'DII activation in one situation, but is without effect in the other is presently not clear. This phenomenon will be further dealt with in the discussion.

While a number of components contribute to energy expenditure, in higher animals the other side of the energy balance equation consists of a single variable- that of food intake. Although feeding behavior is now generally regarded as a regulated entity, the mechanisms through which this regulation is accomplished are not yet well understood. Certainly a large number of variables, both central and peripheral, appear to be involved. A role for the hypothalamus in the central control of food intake was first

suggested more than 100 years ago, with a description of obesity related to a brain tumor in the region of this structure (Mohr, 1840 in Morgane, 1979). Nearly 100 years later, lesions of the hypothalamus were first conclusively established to result in obesity (Bailey and Bremer, 1921). Subsequent observation that electrolytic destruction of the ventromedial hypothalamus (VMH) resulted in a syndrome of hyperphagia and obesity (Hetherington and Ranson, 1939,1940) and that lesions of the lateral hypothalamus resulted in reduction of food intake (Anand et al, 1951) formed the basis for the "dual center hypothesis" (Stellar 1954). This theory proposed that the motivation "to eat or not to eat" resided in a "feeding center" in the lateral hypothalamus and a "satiety center" in the VMH, and that the amount of feeding behavior was a function of the amount of activity built up in these areas. The observations that electrical stimulation of the VMH could suppress feeding in hungry rats (Anand and Dua, 1955), while stimulation of the LH could cause feeding in satieted rats were seen as supporting this idea.

Over the last 10 years, a number of observations have suggested that Stellar's dual-center theory is overly simplistic. For one thing, alterations in feeding behavior produced by lesioning cannot be unequivocally attributed to actions of the hypothalamus, since this structure is the site of many pathways and "circuits" to and from other brain

areas (Morgane, 1979). Similarly, stimulation of regions outside the hypothalamus can give rise to the same types of changes that result from hypothalamic stimulation (Sclafani and Kirchgessner, in press). Thus, the dual center hypothesis has given way to other theories which suggest a less autonomous role of the hypothalamus in the regulation of food intake, and a greater involvement of surrounding brain areas. Currently, the hypothalamus is viewed as an important integrating and relay station for coordination of signals arriving from the periphery (chemo- and stretch-receptor signals from the gut, neural and metabolic signals from the liver, and hormonal and metabolic signals from the circulation) and from higher brain centers (Morgane, 1954, Sclafani and Kirchgessner, in press).

While a well-defined syndrome of hyperphagia and obesity resulting from medial hypothalamic (MH) lesions has in the past been attributed to destruction of the ventromedial nucleus (VMN) (for reviews see Sclafani, 1984, Sclafani and Kirschgessner, in press), more recent experiments employing precise knife-cut techniques have suggested that MH lesions produce hyperphagia by destroying fibers of passage just lateral to this nucleus (Sclafani and Kirschgessner, in press). These fibers are proposed to be part of a common "feeding inhibitory pathway", which passes through the perifornical region of the hypothalamus, turns medially just rostral to the VMN, and takes an as-yet ill-defined path throught the brainstem. The hypothalamic focus

of this pathway may in fact be not the VMH, but rather the paraventricular nucleus (PVN) (Gold et al, 1977). In its course through the brainstem, the putative feeding-inhibitory path is believed to pass through the nucleus of the solitary tract, the principal recipient of gustatory and visceral afferent information (Torvik, 1956, Beckstead and Norgren, 1979, Leslie et al, 1982). While the exact terminus of the pathway is not yet determined, the nucleus of the solitary tract and the dorsal motor nucleus of the vagus are implicated in this role (Swanson and Kuypers, 1980, Swanson and Sawchenko, 1983). Based on its structural organization, it has been suggested that the PVN, with input from other centers, may regulate feeding activity via the feeding-inhibitory path by modulation of visceral and gustatory afferent information at the level of the solitary tract nucleus, and by regulation of sympathetic and parasympathetic activity through its connections with the spinal cord and the dorsal motor nucleus of the vagus (Sclafani and Kirchgessner, in press).

Thus, it is possible that the various obesity-promoting manipulations of the VMH, including electrolytic, knife cut and chemical lesions, result from destruction of the feeding-inhibitory pathway. However, the functional sequence of events between lesioning and production of hyperphagia is still far from clear. Some workers attribute hyperphagia to a deficit in short-term satiety (for review

see Storlien, 1985). Others have suggested that overeating results from an alteration in response to the orosensory properties of food; this suggestion is based upon an enhanced overeating response to "tasty" or "palatable" foods and an exaggerated undereating response to unpalatable foods in VMH-lesioned animals (for reviews see Graff and Stellar, 1962, Le Magnen, 1983, 1984, Storlien, 1985). A third theory, termed the "autonomic-metabolic hypothesis", essentially suggests that the VMH lesion instigates an altered autonomic outflow which in turn produces a neuroendocrine state conducive to overeating (Bray and York, 1979, Le Magnen, 1983, Storlien 1985). This theory is supported by the occurrence of a characteristic hyperinsulinemia in VMH-lesioned animals (Hales and Kennedy, 1964), by the fact that vagotomy blocks their hyperphagia, hyperinsulinemia and obesity (Powley and Opsahl, 1974), and also because lesion-induced reduction of sympathetic activity is observed (Vander Tuig et al, 1982). The second and third theories have been combined as the "cephalic phase hypothesis" (Powley, 1977), which proposes that VMH lesions produce their major effects on feeding behavior by heightening autonomic and endocrine responses triggered by oropharyngeal contact with the food. Each of the four theories presented here comes with many supporting and contradictory observations; a more satisfying characterization of the sequence of events between destruc-

tion of the feeding inhibitory pathway and the occurrence of hyperphagia awaits further study. Furthermore, the relationship between this pathway and other putative central feeding-related systems (for references see Sclafani and Kirchgessner, in press) remains to be clarified.

Among the many methods which have been employed for the production of hypothalamic obesity is that of the goldthioglucose (GTG) lesion. GTG administered intraperitoneally was first shown to cause hyperphagia and obesity in mice more than thirty years ago (Brecher and Waxler, 1949). Evidence for a hypothalamic involvement in the mechanism of action was obtained shortly thereafter, with the demonstration of extensive hypothalamic damage in mice examined 2-3 days after GTG administration (Marshall et al, 1955). Further study revealed that success in producing the GTG obesity syndrome is dependent upon dose, with a moderately reproducible relationship between incidence of obesity and mortality rate (Liebelt et al, 1960, 1966). The degree of obesity attained by lesioned animals is influenced by species and genetic background (Brown and Viles, 1983, Liebelt et al, 1960).

The administration of a necrotizing dose of GTG produces a characteristic syndrome of obesity, which results from abnormalities in both energy intake and energy expenditure. This syndrome can be roughly divided into three stages. A first stage of anorexia and weight loss lasts for 3-7 days

(Debons,et al, 1982, De Laey et al; 1975) and is attributed to the acute toxic effects of the drug. The initial weight loss is succeeded by a "dynamic" phase that is distinguished by weight gain and hyperphagia, and may persist for four to six weeks. During this period, GTG-obese mice display the following characteristics: serum levels of corticosterone are normal (Saito and Bray, 1983) and mice retain an essentially normal glycemia and glucose disposal (Le Marchand et al, 1978); however signs of impending insulin resistance are present, including mild hyperinsulinemia and slightly impaired muscle response to insulin (Le Marchand et al, 1978). Approximately six weeks after GTG administration, mice enter the final "static" phase of the obesity syndrome, at which time body weight stabilizes at double or more the weight of controls, and food intake returns to normal levels (Gray and Liebelt, 1961). The static phase is associated with marked abnormalities in glucose regulation, including hyperglycemia, hyperinsulinemia and insulin resistance (Le Marchand et al, 1978). Blood corticosterone levels are elevated, but only in the morning hours (Saito and Bray, 1983).

When considered on an overall basis, GTG-obese mice have a faster growth rate than unlesioned animals; this is reflected in a greater naso-anal length and increased body protein (Hogan and Himms-Hagen, 1983, Brecher and Enger, 1954, in Sinha et al, 1975). As well, they have a greater

bone mass (De Leeuw, et al, 1981). The cause of their faster growth is not known. Serum levels of growth hormone are reduced in GTG-obese mice, but stimulated secretion may be greater than normal (Sinha et al, 1975) and thus may be responsible for the increased size of the mouse. Based on thyroidal ^{125}I uptake and release techniques, thyroid activity in GTG-obese mice has been reported as normal in all phases of obesity (Schindler and Liebelt, 1967). Serum thyroid hormone levels have not been studied.

The anatomical extent and severity of damage caused by GTG-administration varies between individual animals, but in most cases several hypothalamic regions are involved. Thus, GTG-induced lesions have been reported in the VMN, supraoptic nucleus (SN), the ventral part of the anterior and lateral hypothalamic area, the arcuate nucleus (AN), and the median eminence (Marshall et al, 1955, Debons et al, 1970a, Kataoka et al, 1978). In addition, significant damage has been reported at extrahypothalamic sites including the area above the optic chiasm, the hippocampal commissure, the hindbrain at the level of the vestibular nuclei in the floor of the fourth ventricle, and in neurons contiguous with the area postrema, including those in the visceral sensory and dorsal motor nuclei of the vagus (Swartz et al, 1960, Debons et al, 1962, Kataoka et al, 1978, Powley and Prechtl, 1986).

The mechanism by which GTG gives rise to selective

lesions and obesity is not resolved. Mayer (1955) and others (Luby et al, 1981, for example) have proposed that GTG is concentrated at lesion sites by binding of the glucose moiety to specific hypothalamic glucoreceptors. A large number of observations support this notion: hypothalamic neurons which change their rate of firing in response to altered glucose levels have been identified (Anand et al, 1964, Oomura and Yoshimatsu, 1984); the glucose moiety of GTG is essential for production of hypothalamic lesions- other gold-thio compounds (eg gold-thiomalate, gold-thiosorbitol) are readily taken up by brain tissue, but fail to cause necrosis, hyperphagia or obesity (Mayer, 1960); similarly, after GTG injection, gold is found throughout the brain and also in non-brain regions, yet lesions are confined to the sites described above (Debons et al, 1970a, 1970b); compounds which inhibit glucose uptake, such as phlorizin (Brown and Viles, 1982), and 2-deoxy-glucose (Likuski et al, 1967) prevent GTG necrosis; moreover, GTG lesions are blocked in streptozotocin-diabetic animals, while sensitivity is restored when these mice are supplemented with insulin (Debons et al, 1968,1969). In fact, studies of the mechanism of action of GTG have indicated that in contrast to the brain as a whole, in the hypothalamus and certain extrahypothalamic regions there is an insulin-dependent mechanism for uptake of glucose.

While the initial effects of GTG damage are thought to center around specific glucoreceptor neurons, the regions which eventually undergo necrosis are functionally and anatomically heterogenous (Debons et al, 1979a). It has been suggested that initial uptake of GTG by glucose sensitive neurons results in release of serotonin, which in turn causes damage to adjacent capillaries (Debons et al, 1979, and references therein). Vascular damage and the resulting ischemia are thought to be responsible for the necrosis of surrounding hypothalamic regions.

While damage to hypothalamic glucoreceptive neurons has been proposed to account for the altered feeding behavior of the GTG-obese mouse, it may be that the hyperphagia of this animal is due to destruction of the putative feeding-inhibitory pathway. Certainly, lesions which may have their focal point in the VMN nonetheless extend into adjacent regions, through which this path is thought to run. As well, at least two extrahypothalamic sites of GTG-induced damage occur in brain regions which appear to be important components of the feeding inhibitory pathway- these are the nucleus of the solitary tract and the dorsal motor nucleus of the vagus (Powley and Prechtl, 1986). It is of note that the degree of obesity attained by lesioned animals has been correlated with the extent of hypothalamic damage (Liebelt et al, 1960, 1966). This finding may indicate either that a large lesion causes greater damage to an anatomically

diffuse pathway of feeding-inhibitory neurons, or that a large lesion causes damage of more than one system involved in the control of food intake. A number of systems in addition to the feeding-inhibitory pathway are thought to exist (Sclafani and Kirchgessner, in press).

Although hyperphagia was for many years regarded as the primary cause of obesity in the GTG-lesioned mouse, the increased food intake demonstrated by these animals does not entirely account for their enhanced weight gain. The duration and magnitude of hyperphagia in GTG-obesity is variable, and appears to be influenced by a number of factors, including type of diet, environmental temperature, and age of animals when lesioned (De Laey et al, 1975). Although successfully-lesioned mice invariably become obese the period of hyperphagia may range from as little as 3-4 days (De Laey et al, 1975) to as long as a month (Djazayery et al, 1979). In fact, when considered on a cumulative basis, and accounting for greater body size, GTG-obese mice are sometimes reported as normo- or even slightly hypophagic (Djazayery et al, 1979). Moreover, GTG-lesioned mice fed a restricted diet gain more weight and become more obese than unlesioned animals fed the same amount of food (Zaror-Behrens and Himms-Hagen, 1984). Therefore, the obesity of these animals must be ascribed not only to increased food intake, but also to a decreased energy expenditure. Indeed, GTG-obese mice housed at 27 °C are reported to have a

metabolic efficiency that is four times that of unlesioned mice, and a significantly lower oxygen consumption when expressed per metabolic body weight (Djazayery et al, 1979). Thus, it is plain that the obesity of this animal develops not only from its hyperphagia, but from alterations in both sides of the energy balance equation.

In all studied models of genetic or hypothalamic obesity (ob/ob mouse, fa/fa rat, VMH-lesioned rat), increased metabolic efficiency has been associated with defective control of BAT thermogenesis or growth (Himms-Hagen, 1985). Such a defect has also been suggested in the GTG-obese mouse. In the dynamic phase of its obesity, the BAT of this animal is relatively inactive at temperatures close to thermoneutrality (Hogan and Himms-Hagen, 1983). During the same period, GTG-obese mice have been found not to activate either BAT thermogenesis or sympathetic activity in BAT when fed a palatable cafeteria diet (Hogan and Himms-Hagen, 1983, Zaror-Behrens and Himms-Hagen, 1984). Thus, a lower basal activity of BAT and a reduced diet-induced thermogenesis are believed to contribute to the high metabolic efficiency and obesity of the GTG-lesioned mouse.

The cause of the apparent lower basal thermogenic activity in BAT of the GTG-obese mouse is unknown. One possibility is that this characteristic is maintained through an action of the adrenal glucocorticoids, since corticosterone is known to have a suppressive effect on BAT

thermogenesis. In lean mice, systemic administration of large doses of corticosterone results in reduced GDP-binding to isolated BAT mitochondria, meanwhile producing the hyperphagia, obesity and increased metabolic efficiency typical of genetically obese or lesioned animals (Galpin et al, 1983). In the genetically obese (ob/ob) mouse, elevated serum corticosterone levels are associated with low thermogenic activation of BAT; adrenalectomy produces an elevated GDP-binding in this animal (Holt and York, 1984). As well, BAT thermogenic function is improved by adrenalectomy in the fa/fa Zucker rat (Holt et al, 1983).

The mechanism by which corticosterone mediates suppression of BAT thermogenesis is not well defined, but may involve a central effect upon sympathatic nervous system activity, since adrenalectomy has been shown to increase the normally low noradrenaline turnover rate of ob/ob and fa/fa animals (Vander Tuig et al, 1984, York et al, 1985). As well, this effect appears to be specific for the center controlling diet-induced thermogenesis, since cold-induced BAT thermogenesis occurs normally in corticosterone-treated animals (Galpin et al, 1983).

In contrast to the genetically obese mouse, but similar to the fa/fa rat, corticosterone levels remain normal in the GTG-obese mouse until its obesity is well advanced (Saito and Bray, 1983). Nonetheless, the adrenal glucocorticoids are essential for the development and maintenance of obesity

in this animal. In the dynamic stage, adrenalectomy (Debons et al, 1982) and hypophysectomy (Powley and Plocker, 1980, Debons et al, 1982b) block the hyperphagia, weight gain and obesity produced by the GTG lesion, while in the static phase, adrenalectomy produces anorexia, weight loss, and eventually death (Debons et al, 1983). In both cases, hyperphagia and obesity are restored by intraperitoneal administration of corticosterone or other glucocorticoids, though not by desoxycorticosterone (Debons et al, 1982). Similar levels of supplementation are without effect on food intake or weight gain of unlesioned adrenalectomized animals (Debons-et al, 1982). Thus, although corticosterone levels are essentially normal in the GTG-lesioned mouse during the period of rapid weight gain, the above observations suggest that an abnormally high sensitivity to the glucocorticoids could be present in this animal. Again, the site of action of corticosterone appears to reside in the central nervous system, since intracerebroventricular administration of minute amounts of this hormone restores the hyperphagia and obesity of adrenalectomized GTG-obese mice, whereas systemic injection of similar amounts does not (Debons et al, 1986). It is possible that a central sensitivity to the adrenal glucorticoids is normally antagonized at the level of the VMH, and that this antagonism is absent in the GTG-lesioned mouse (Himms-Hagen, 1985a). If this is the case, suppression of BAT thermogenesis could occur through the

action of corticosterone, notwithstanding the normal serum levels of this hormone, and could result in the reduced body temperatures observed in food-restricted GTG-obese animals. Similarly, enhanced sensitivity to corticosterone has been reported in the fa/fa Zucker rat (Freedman et al, 1986) and the ob/ob mouse (Tokuyama and Himms-Hagen, 1987) and is associated with abnormal thermogenic function in these animals.

With respect to the abnormal DIT of the cafeteria-fed GTG-obese mouse, the site of the defect is presumably at the level of the lesioned hypothalamus, and has been proposed to result from an interruption of neural pathways between the reception of signal derived from the diet and sympathetic innervation of BAT (Hogan and Himms-Hagen, 1983). The tissue itself appears to be undamaged, since isolated BAT mitochondria have a normal ultrastructure and can undergo thermogenic activation in the cold (Hogan and Himms-Hagen, 1983). However, BAT of the GTG-obese mouse grows normally with cafeteria feeding, and in fact develops a greater mass than that of control. This observation is surprising, since sympathetic stimulation, usually considered to be the primary stimulus of BAT growth (Desautels and Himms-Hagen, 1979), appears to be absent in the cafeteria-fed GTG-obese mouse (Zaror-Behrens and Himms-Hagen, 1984). It is conceivable that the larger mass of BAT is a consequence of the overall faster growth of this animal. However, 3 weeks

of cafeteria feeding has been shown to increase the overall growth rate of both lean and GTG-obese mice by approximately 2%, whereas BAT protein is increased by 24% in lean mice and 35% in GTG-obese mice (Hogan and Himms-Hagen, 1983). The above explanation therefore does not account for the effect of cafeteria feeding to increase the size of BAT above that seen in chow fed GTG-obese mice. Alternatively, it may be that sympathetic stimulation in BAT of the GTG-obese mouse is activated by cafeteria feeding, but only for part of each day. Reported measurements of thermogenic and sympathetic activation have all been performed in the early part of the light phase, when thermogenic activity is expected to be at a minimum (Rothwell and Stock, 1983).

Part of the increased metabolic efficiency of the GTG-obese mouse appears to result from its ability to thermoregulate at a reduced body temperature when fed chow or restricted diet. Thus, these animals maintain a lower body temperature than their lean counterparts for a large proportion of each day. While the reduced basal thermogenic activity in BAT is likely to account for at least part of the hypothermia in chow-fed GTG-obese mice, the involvement of BAT in the altered thermoregulation of food-restricted GTG-obese mice has not been characterized.

Because of the high metabolic efficiency observed in GTG-obese mice, it is surprising that these animals have been described as "resistant" to torpor (Webb et al, 1982).

GTG-obese mice have been reported to maintain euthermia even when deprived of food for 48 hours, while under the same conditions, lean mice enter torpor readily (Webb et al, 1982). However, in this report, mice were normally fed ad libitum, and were obese at the time of the fast. More recently it has been observed that GTG-obese mice fed a restricted amount of diet in the early afternoon display a regular morning torpor, as do lean mice fed in the same way (Zaror-Behrens and Himms-Hagen, submitted). In this study, restricted feeding was begun immediately after lesioning, therefore minimizing the development of obesity. As well, temperature recordings were performed after 3 weeks of restricted feeding. Thus, there are two possible explanations for the discrepancy between these reports. First, the tendency of the GTG-obese mouse to become torpid could well depend on its degree of obesity. As with unlesioned animals, restricted-fed GTG-obese mice may enter torpor to conserve their limited energy reserves, while obese animals could rely instead upon their large fat stores to supply requirments during a fast, as the same time maintaining euthermia. Resistance of obese GTG-lesioned mice to torpor would be in contrast to the ob/ob mouse, which abandons euthermia regularly though massively obese, and does so even in the presence of food (Webb et al, 1982). A second possibility is that GTG-obese mice may require a period of entrainment to a limited food supply in order to

exhibit torpor. This period would be provided by a restricted feeding regime, but not by a 48 hour fast. In any case, the demonstration of normally-occurring torpor in restricted-fed GTG-obese mice indicates that the mechanisms involved in its control are intact, and in appropriate circumstances are functional in this animal.

Thermoregulation has in the past been regarded as normal in the GTG-obese mouse. Thus this animal maintains its body temperature and activates BAT thermogenesis when exposed to mild cold (8 °C), (Hogan and Himms-Hagen, 1983) and demonstrates a normal growth of the tissue during acclimation to this temperature. Moreover, GTG-obese mice have been reported to survive acclimation to more severe cold stimulus (4 °C) (Davis and Mayer, 1954). However, though present, BAT thermogenic activation is lower than normal in the cold-exposed GTG-obese mouse. This observation, and the previously-discussed demonstration of lower daily body temperatures in this animal, suggests that a defect or alteration of thermoregulation may be present in the GTG-obese mouse.

STATEMENT OF THE PROBLEM

From the preceding discussion, it should be clear that the obesity of the GTG-lesioned mouse results from abnormalities of both energy intake and energy expenditure. The latter component most certainly involves alterations in the activity of BAT in this animal. Based upon the reviewed findings, the objectives of the present work were the following:

1. DIT is reportedly defective in the GTG-obese mouse, an anomaly which is believed to contribute to its high metabolic efficiency and obesity. Moreover, diet-induced sympathetic stimulation has not been detected in this animal. Surprisingly, cafeteria feeding promotes a normal growth of BAT in the GTG-obese mouse.

Previous studies have all been carried out in the early part of the light phase, when thermogenic activity is likely to be at a minimum. Thus, it was the aim of the present work to see if sympathetic stimulation and DIT occur in the GTG-obese mouse at times not hitherto measured. Such a finding would indicate that the reduced DIT of this animal results from an altered control of this component of thermogenesis, rather than from destruction of the neural pathways through which it is mediated.

The first objective of this work, therefore, was to assess the thermogenic state of BAT in cafeteria-fed GTG-

obese mice at several times of day. A related objective was to characterize the circadian rhythm of BAT thermogenic activity, not previously reported for mice.

2. GTG-obese mice fed a restricted diet gain more weight and become more obese than unlesioned animals fed the same amount of food. Part of the metabolic efficiency of these animals appears to result from their ability to thermoregulate at a reduced body temperature for a large proportion of each day. The contribution of an altered BAT thermogenic activity to the lower body temperature and high metabolic efficiency of the food-restricted GTG-obese mouse has not been characterized.

Thus, the second objective of this study was to assess the thermogenic state of BAT of food-restricted GTG-obese mice at various times of day, specifically during torpor and arousal from torpor. A subsidiary goal was to further characterize the role of BAT in the arousal process.

3. Although thermoregulation has in the past been regarded as normal in the GTG-obese mouse, the maintenance of a reduced daily body temperature, and a smaller-than-normal cold-induced activation of BAT thermogenesis suggests that a defect or alteration of thermoregulation may be present in this animal.

The third objective of this study was to compare the time-course of activation of BAT thermogenesis in lean and GTG-obese mice, to determine whether the lesser activation

at the single time measured previously was due to a lower peak (reduced thermogenic capacity), or a displaced peak (delayed thermogenic activation).

4. The final objective of this work was to introduce into the above studies an examination of the activity of BAT thyroxine 5'-deiodinase, and thus to characterize the effects of dietary manipulation and cold treatment on the activity of this enzyme in mice. Since a marked reduction in the cold-induced activity of 5'-DII has been demonstrated in the genetically obese (ob/ob) mouse and the Zucker (fa/fa) rat, a related goal was to find out whether a similar defect might occur after GTG-induced hypothalamic lesioning.

CHAPTER II

MATERIALS AND METHODS

PART I: MATERIALS

A: Animals

Female mice (lean homozygous, +/+) were obtained from the C57BL/6J colony at Jackson Laboratories, Bar Harbor, ME. Experimental procedures were begun after mice had been acclimated to holding conditions for at least two weeks. Unless otherwise specified animals were housed at 26.5 °C in plastic cages with wood chip bedding, and had free access to food (Purina Rat Chow) and water. Normally mice were kept in groups of three or four but were placed in individual cages for studies carried out at 4 or 14 °C. In all experiments animals were maintained on a controlled lighting schedule (12L:12D, lights on at 0600 hours).

B: CHEMICALS

Biochemicals, buffers, acids and other common reagents were purchased from Sigma Chemical Co. and Fisher Scientific. [U-^{14}C]-sucrose, 20.70 GBq/mmol, (560 mCi/mmol) was obtained from ICN Biomedicals Canada. Other radiochemicals were purchased from Amersham: [8-^{3}H]guanosine 5'-diphosphate, ammonium salt, 418 GBq/mmol (11.3Ci/mmol), [3',5'-^{125}I]thyroxine, > 44 MBq/µg

(1200μCi/μG) thyroxine, Amerlex™ total T_3 and T_4 RIA kits. Cation exchange resin (AG 50 W-X2 100-200 mesh) for the thyroxine 5'-deiodinase assay was obtained from Biorad. Thyroid hormone-free normal human serum was purchased from AMF Biologicals and Diagnostic Co.

C: DIETS

1. **Cafeteria Diet** Cafeteria-fed animals were offered five types of food presented in three different revolving menus. Each menu consisted of Purina Rat Chow, chocolate wafers, nuts, cheese and cookies. The type of nut, cheese, or cookie was varied daily. Allotments of cafeteria diet were provided at 1500h, and amounts were sufficient that animals could choose any food type ad libitum. Further descriptions of cafeteria menus and nutrient composition of the various food items are given in appendix B.

2. **Restricted Diet** Animals receiving the restricted diet were fed Purina Rat Chow at 60% of the ad libitum level of intake of lean, chow-fed controls. This level was previously shown to minimize the development of obesity in GTG-lesioned mice and to promote early morning torpor in lean and GTG-obese mice (Himms-Hagen, 1985, Zaror-Behrens and Himms-Hagen, submitted). All animals received their rations each day at 1500h. This time was chosen rather than

a point during the dark phase so that the effect of the meal on regulation of body temperature and thermogenic state of BAT could be examined independently of the effects of the lighting schedule.

PART II: METHODS

A: GTG LESION

Animals arrived at 7-9 weeks of age and were housed as described for two weeks prior to injection. At 9-11 weeks of age, all animals were fasted overnight. In the morning, mice chosen for GTG treatment were injected intraperitoneally with a 7% solution of goldthioglucose (aurothioglucose, Sigma) in 0.9% saline. Food was returned to animals immediately after injection. The dosage used for all experiments was 700 mg GTG/kg body weight. This level was expected to give a survival rate of 90% and an incidence of obesity of 65% (Hogan and Himms-Hagen, 1983, de Laey et al, 1975). Success of lesioning was confirmed by measurement of growth rate. Animals were considered to be "GTG-obese" if their rate of body weight gain 3-4 weeks after injection exceeded three times the rate observed in lean control mice.

B: MEASUREMENT OF FOOD INTAKE

Measurement of 24-hour food intake was performed twice

per menu for cafeteria-fed animals and twice per week for chow-fed mice. Weighed amounts of food were given at the usual feeding time and the uneaten portion was collected twenty-four hours later. Uneaten food was weighed after a short drying period and intake was calculated by difference, with correction made for loss of moisture in some foods. Since animals were housed in groups of varying numbers (usually 3 or 4) mean caloric intakes were calculated per mouse per cage, using published values for caloric content and nutrient composition of the different foods (Pennington and Church, 1980, see appendix B). These values were also used to determine the proportions of protein, fat and carbohydrate consumed during each intake study. During food intakes, cafeteria-fed mice were housed without bedding to facilitate collection of uneaten food. Chow-fed animals had the usual wood-chip bedding during these studies.

C: TEMPERATURE MEASUREMENTS

For all experiments, rectal temperatures were measured at the time of sacrifice with a Bailey Instruments digital thermocouple thermometer, model BAT-8, with a flexible Teflon-sheathed microprobe. For experiment 1, temperatures were also recorded repeatedly over a two week period, at eight different time points. Disturbance of mice at the time of recording was minimized as much as possible and no more than one measurement was made on any animal in a 24

hour period. During the dark phase (1800h-0600h) a dim incandescent light was used. For experiment 3, temperatures were recorded in the manner described above, except that measurements were made on each mouse at hourly intervals until animals were removed from the cold.

D: ISOLATION OF BAT MITOCHONDRIA

Disturbance of animals prior to sacrifice was avoided as much as possible, to minimize sympathetic stimulation unrelated to experimental treatments (Depocas and Behrens, 1977). Studies conducted during the dark phase were performed using a dim incandescent lamp. Animals were sacrificed by decaptiation, and blood was collected into chilled Eppendorf microcentrifuge tubes. Inter- and subscapular BAT were quickly removed and placed in ice-cold isolation medium containing 0.25 M sucrose, 0.2 mM EDTA, (dipotassium salt), and 1 mM HEPES, adjusted to pH 7.2 with 1 M KOH. Tissues were cleaned, weighed, minced with scissors, and homogenized with isolation medium, using four strokes of a glass/teflon homogenizer at 400 rmp. Homogenates from each animal were diluted to ten ml, and aliquots were taken for later determination of protein and thyroxine 5'-deiodinase activity. Remaining homogenates were diluted to 42 ml, and centrifuged for ten minutes at 3000 rpm, using an HB-4 rotor in a refrigerated centrifuge (Sorvall RC-5B, brake off). Subsequent isolation of BAT

mitochondria was carried out as described by Slinde et al (1975), except that sedimentation of combined supernatants was performed in 14, rather than in 42 ml tubes. Final mitochondrial pellets were resuspended in a volume of isolation medium suitable to give a protein concentration of approximately 5 mg/ml. Mitochondrial suspensions were used immediately for measurement of GDP binding. All samples were maintained on ice throughout the isolation procedure.

E: PROCEDURE FOR MEASUREMENT OF GDP BINDING

The purine nucleotide binding assay developed by Nicholls (1976) and modified by Desautels et al (1978) was used to measure binding of [^{3}H]-GDP to BAT mitochondria. Twenty µl of freshly isolated mitochondria (approximately 5 mg protein/ml) were incubated for two minutes in the presence or absence of 1 mM ADP in a mixture containing 1mM EDTA (disodium salt) 10 mM choline chloride, 20 mM TES, 100 mM sucrose, 4.7 µM rotenone, 100 µM atractyloside (sodium salt), 0.19 µCi/ml [^{14}C]-sucrose, and 0.72 µCi/ml [^{3}H]-GDP, with a total concentration of 10 µM GDP. After incubation samples were centrifuged for 2 minutes at 12000 rpm in an Eppendorf microcentrifuge. The supernatant was aspirated and mitochondrial pellets were dissolved by incubation with NCS tissue solubilizer for 12 hours. Samples were counted with a Beckman LS 6800 liquid scintillation counter in a cocktail consisting of 0.05 ml of 10% ascorbic acid, and 10

ml of toluene with 0.65% PPO. Specific binding of GDP was calculated as the difference in binding between samples incubated in the presence or absence of ADP, and was expressed as pmol bound/mg mitochondrial protein. Correction for differences in volume of incubation medium trapped within mitochondrial pellets was made using values obtained for [^{14}C]-sucrose.

F: PROTEIN ESTIMATION

BAT homogenates were precipitated in ice-cold, 12.5% trichloroacetic acid, and were stored at 4 °C for at least 24 hours, or for up to two weeks. Samples were centrifuged at 4200 rpm for 20 minutes using a Sorvall J-6B centrifuge with 4.2 rotor. Supernatants were removed by aspiration, and pellets were dissolved in 0.5% NaOH, for 15 minutes at 37 °C. Protein contents were estimated using the Lowry method (Lowry et al, 1951) as modified by Schacterle and Pollack (1973), using bovine serum albumin as the standard. Mitochondrial protein determinations were carried out in a similar manner, except that precipitation steps were omitted. Samples of final mitochondrial suspensions were added to 0.5% NaOH and the modified Lowry assay was performed. Volumes of isolation medium equal to volumes of mitochondrial samples were added to BSA standards. This addition corrected for the contribution of isolation medium

in mitochondrial suspensions to measured absorbance readings.

G: MEASUREMENT OF THYROXINE 5'-DEIODINASE ACTIVITY IN BAT

BAT samples were homogenized with four strokes of a glass/teflon homogenizer at 400 rpm, followed by twenty strokes (by hand) of an all-glass homogenizer. Samples were frozen in liquid nitrogen and were stored at -80 °C for up to four weeks; under these conditions thyroxine 5'-deiodinase is stable for at least four months (Kates and Himms-Hagen, 1985). Enzyme activity was measured essentially as described by others (Visser at al, 1982, Leonard and Rosenberg, 1980, Leonard et al, 1983), using conditions established for mouse BAT (Kates and Himms-Hagen, 1985). Measurement of enzyme activity was based on the release of radioiodine from ^{125}I-labelled T_4. Samples were incubated under nitrogen for 30 minutes at 37 °C, in a reaction mixture containing 1mM EDTA (pH 7.0), 10 mM dithiothreitol (DTT), 1mM propyl-2-thiouracil (PTU), approximately 0.15 nM ^{125}I-T_4 (to give 50,000 cpm per sample), plus 2.32 nM unlabelled T_4, with a total thyroxine concentration of about 2.57 nM. Each assay tube contained 30-100 µg homogenate protein; at these concentrations the rate of ^{125}I liberation was linearly related to protein content (Kates and Himms-Hagen, 1985). Reactions were

terminated by binding the majority of substrate using 50 µl of ice cold thyroid hormone-free normal human serum, containing T_3 and T_4 binding proteins. Protein-bound substrate was precipitated with 350 µl of ice-cold 12.5% TCA, followed by centrifugation for 2 minutes at 14000 rpm (Eppendorf microfuge, room temperature). Free radioiodine was separated from remaining substrate by passing 500 µl of supernatant through a Dowex AG 50 W-X2 100-200 cation exchange column (1.2 ml bed volume) equilibrated in 10% acetic acid. ^{125}I was eluted with 3- 1 ml washes of 10% acetic acid, and radioactivity was measured using a Beckman gamma 4000 counter. Radiolabelled thyroxine was purified immediately prior to use in these asays by paper electrophoresis at pH 7.1. ^{125}I-T_4 was applied to Whatman 3mm filter paper strips (2 x 20 cm) and electrophoresis was carried out in 50 mM ammonium acetate at 25 V/cm. Under these conditions, free radioiodine travelled 4-6 cm in 10 minutes, while ^{125}I-thyroxine remained at the origin. T_4 was extracted after electrophoresis using methanol:ammonia (99:1) (Kaplan and Yaskoski, 1980). Purification resulted in a free iodine content of less than 1%. Correction for non-enzymatic deiodination was made by subtraction of values obtained from control tubes from those obtained for samples. Control tubes were incubated without homogenate protein, with the total assay volume made up using buffer.

H: MEASUREMENT OF THYROID HORMONES IN SERUM

Blood was collected from mice after decapitation and was maintained at 4 °C for up to three hours. Samples were centrifuged (Eppendorf microfuge) for 15 minutes at 14000 rpm. Serum was collected and was stored at -20 °C prior to thyroid hormone estimation. Total T_3 and T_4 were measured using commercially available radioimmunoassay kits (Amerlex^{TM} total T_3 or total T_4 RIA kits, Amersham Corporation). With these kits, measurement is based on competition between serum hormone and ^{125}I-T_3 or ^{125}I-T_4 for sites on a T_3 or T_4 specific antibody. The antibody is bound to polymer particles; separation of T_3 or T_4 bound to the antibody-polymer complex is carried out by centrifugation at 4200 rpm for 20 minutes (J6B centrifuge, 4.2 rotor, brake off). For mouse samples, it was necessary to carefully aspirate the supernatant rather than decanting is since the resulting pellets were easily dislodged. Pellets were then counted in a Beckman Gamma 4000 counter. Levels of serum T_3 or T_4 were determined by comparison with results for thyroid hormone standards in human serum. A set of thyroid control sera samples was included in each incubation batch.

I: STATISTICAL ANALYSIS

Results are expressed as mean ± standard error (SE) of the mean. Statistical analyses were performed using the

SPSS-X21 computer statistics package. Normality of distribution was assessed by measurement of skewness and kurtosis using the Breakdown procedure. Two or three-way analysis of variance was carried out using program ANOVA. Post hoc comparisons were made with simple and simple simple means tests (Kirk, 1968). Repeated comparisons of treatment groups against control means were performed using Dunnett's test.

Results are expressed as significant at $p < .01$ unless otherwise indicated.

CHAPTER III

RESULTS

PART I: BROWN ADIPOSE TISSUE OF GTG-OBESE MICE: EFFECT OF DIET

OBJECTIVES:

Previous studies have demonstrated a defective DIT in BAT of the GTG-obese mouse, and diet-induced sympathetic stimulation has not been detected in this animal. Nonetheless, cafeteria feeding promotes a normal growth of BAT in the GTG-obese mouse. All former experiments have been carried out in the early part of the light phase, when thermogenic activity is likely to be at a minimum.

GTG-obese mice fed restricted diet become more obese than unlesioned animals fed the same amount of food. The contribution of an altered BAT thermogenic activity to the high metabolic efficiency of these animals has not been characterized.

The effects of dietary manipulation upon the activity of BAT 5'-deiodinase have not been reported for mice.

The objectives of the first experiment were thus:

1. To study the thermogenic state of BAT at different times of day in GTG-obese mice fed chow, restricted, or cafeteria diet, to see whether a transient activation had been missed in the former experiments.

2. To characterize circadian changes in BAT thyroxine 5'-deiodinase activity in mice eating different diets, and to assess the effect of GTG-lesioning upon the activity of this enzyme.

METHOD:

Animals were randomly assigned to form two groups with equal mean body weights. At 9-11 weeks of age, one group received a GTG lesion (methods, pp 38). Restricted feeding of some lean and some GTG animals was begun immediately. Cafeteria feeding was begun in other groups of animals one week after lesioning. This period allowed animals to recover from the anorexia which occurred after GTG administration, ensuring that lean and GTG-lesioned animals consumed the cafeteria diet for equal lengths of time. A third group of lean and GTG-lesioned animals, which served as controls, received chow ad libitum. Both restricted and cafeteria-fed groups were given their daily rations between 1400h and 1500h. Food intakes were performed twice for each of the three cafeteria menus, and twice each week for chow-fed animals (methods, pp 38). Beginning three weeks after GTG administration, measurements of rectal temperature were made on all mice (methods, pp 39). By this time animals had received cafeteria diet for two weeks, or restricted or chow diet for three, and unsuccessfully lesioned mice had been excluded from further study on the basis of body weight gain. For cafeteria-fed groups, temperature measurements were taken only when mice were housed with bedding, that is, not during food intakes.

Beginning four weeks after lesioning, animals were killed by decapitation at the following times: cafeteria-

fed mice, 0500h, 1100h, 1500h, 1900h, 2400h; food-restricted mice, 0500h, 0700h, 1100h, 1500h. Chow-fed mice, serving as the controls, were killed at all of the above time points, including 0500h, 0700h, 1100h, 1500h, 1900h, 2400h. Time points were chosen to demonstrate maximum and minimum levels of BAT thermogenic activation in the different groups, as predicted from observed rhythms in rectal temperature (Figures 2a and 2b). Time points for food-restricted mice were also chosen to examine thermogenic activation during arousal from torpor.

After sacrifice, inter- and subscapular BAT was removed, homogenized, and aliquots taken for later determination of protein content and activity of T_4 5'DII. Mitochondria were isolated from the remaining homogenate and measurement of GDP binding was performed. Blood was collected, centrifuged, and serum was stored at -20 °C for later determination of T_3 and T_4 content (methods, pp 45). Gonadal WAT was removed and weighed.

STATISTICAL ANALYSIS:

3-way ANOVA (obesity x diet x time of day at killing) was used for analysis of all variables tested for circadian variation, including GDP binding, (figures 4 and 5), specific and total activity of BAT 5'DII, (figures 5 - 9), and serum thyroid hormone concentrations (figures 10 - 13). At each time point, individual treatment effects (diet and

lesion) were determined using simple simple means analysis (Kirk, 1968). The presence of a circadian variation for a particular variable was assessed by comparing the values observed at each time point to the value obtained at 0500h. For this test, Dunnett's post hoc test for comparison of several treatment groups to a single control (Kirk, 1968), was used. For variables that were not assessed with respect to circadian variation (WAT, BAT, BAT protein, table 1), data were analyzed using 2-way ANOVA (obesity x diet). Individual treatment effects were determined using simple means analysis (Kirk, 1968). Data for food intake (table 2) were analyzed by 3-way repeated measures ANOVA (obesity x diet x duration of feeding) (Winer, 1971), and post hoc tests were the same as those used for the three-way ANOVA described above. Body weights (figures 1a and 1b) were analyzed by 2-way ANOVA (obesity x diet) and simple means analysis at a single time point, 5 weeks after administration of the GTG-lesion. Data for circadian rectal temperature measurements were not analyzed statistically, since it was necessary to perform repeated measures upon some, but not all animals. Thus it was not acceptable to perform either a repeated measures or a single measures ANOVA using this data.

For analysis of variance, data for restricted and cafeteria-fed animals were separated, and each group was compared to control animals sacrificed at the same time

points using separate ANOVAs. This separation was necessary since animals from both treatments did not appear at every time point, thus preventing statistical analysis of the combined data.

The results of this experiment have been presented in two ways. First, in order that visual comparison of circadian rhythms in the different groups can be made, data for all three diet groups are presented together (Figure 4, for example). Secondly, for assessment of the effect of GTG lesion, the results for cafeteria-fed and food-restricted groups, with their respective controls, are presented separately (figures 5a and 5b, for example). It should be noted that the statistical results for circadian rhythm arise from separate analysis of cafeteria- and restricted-fed animals with their controls, and not from the combination of data from all treatment groups.

RESULTS

1. DEVELOPMENT OF OBESITY IN GTG-LESIONED MICE

Injection of goldthioglucose produced a transient anorexia which was reflected in the reduced body weights observed at week one (figures 1a and 1b). Analysis of the body weights 5 weeks after lesioning showed that GTG-obese mice in all groups became significantly heavier than similarly-fed lean mice. For both lean and GTG-obese mice,

FIGURE 1a: WEIGHT CURVE OF GTG-OBESE MICE AND LEAN CONTROLS: EFFECT OF CAFETERIA DIET

Values represent means ± SE for 48 lean and 37 GTG-obese mice fed chow, and 48 lean and 11 GTG-obese mice fed cafeteria diet. Measurements were performed at weekly intervals. The time of lesioning is indicated by the arrow marked **GTG.** Cafeteria feeding was begun at the time marked **CAFE DIET.** **END** signifies average final group weights of mice killed over the two week experimental period, between 6 and 8 weeks after GTG-lesioning. Statistical analyses were performed on results of the week 5 measurement.

SYMBOLS:

-□- **LEAN CHOW-FED**

-■- **LEAN CAFETERIA-FED**

-△- **GTG CHOW-FED**

-▲- **GTG CAFETERIA-FED**

* Significant effect of diet, comparing mice of the same type

◇ Significant effect of GTG treatment, comparing mice eating the same diet

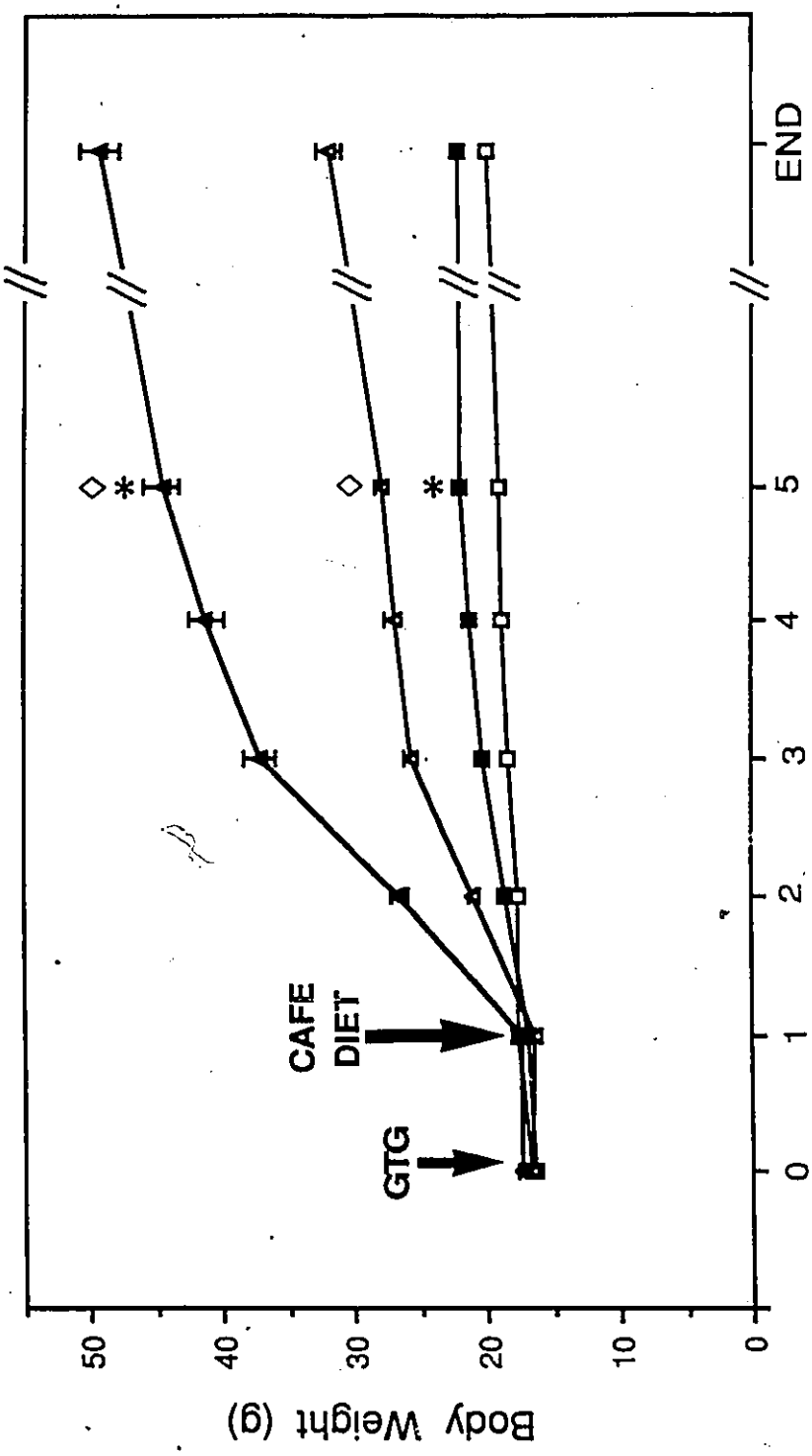

Body Weight (g)
50
40
30
20
10
0
CAFE DIET
GTG
0
1
2
3
4
5
END
WEEKS AFTER GTG LESION

FIGURE 1a

FIGURE 1b: WEIGHT CURVE OF GTG-OBESE MICE AND LEAN CONTROLS: EFFECT OF RESTRICTED DIET

Values represent means ± SE for 48 lean and 37 GTG-obese mice fed chow, and 48 lean and 37 GTG-obese mice fed restricted diet. Food-restriction was begun immediately after lesioning. For further information see legend to figure 1a.

SYMBOLS:

-□- **LEAN CHOW-FED**

-■- **LEAN FOOD-RESTRICTED**

-△- **GTG CHOW-FED**

-▲- **GTG FOOD-RESTRICTED**

* Significant effect of diet, comparing mice of the same type

◇ Significant effect of GTG treatment, comparing mice eating the same diet

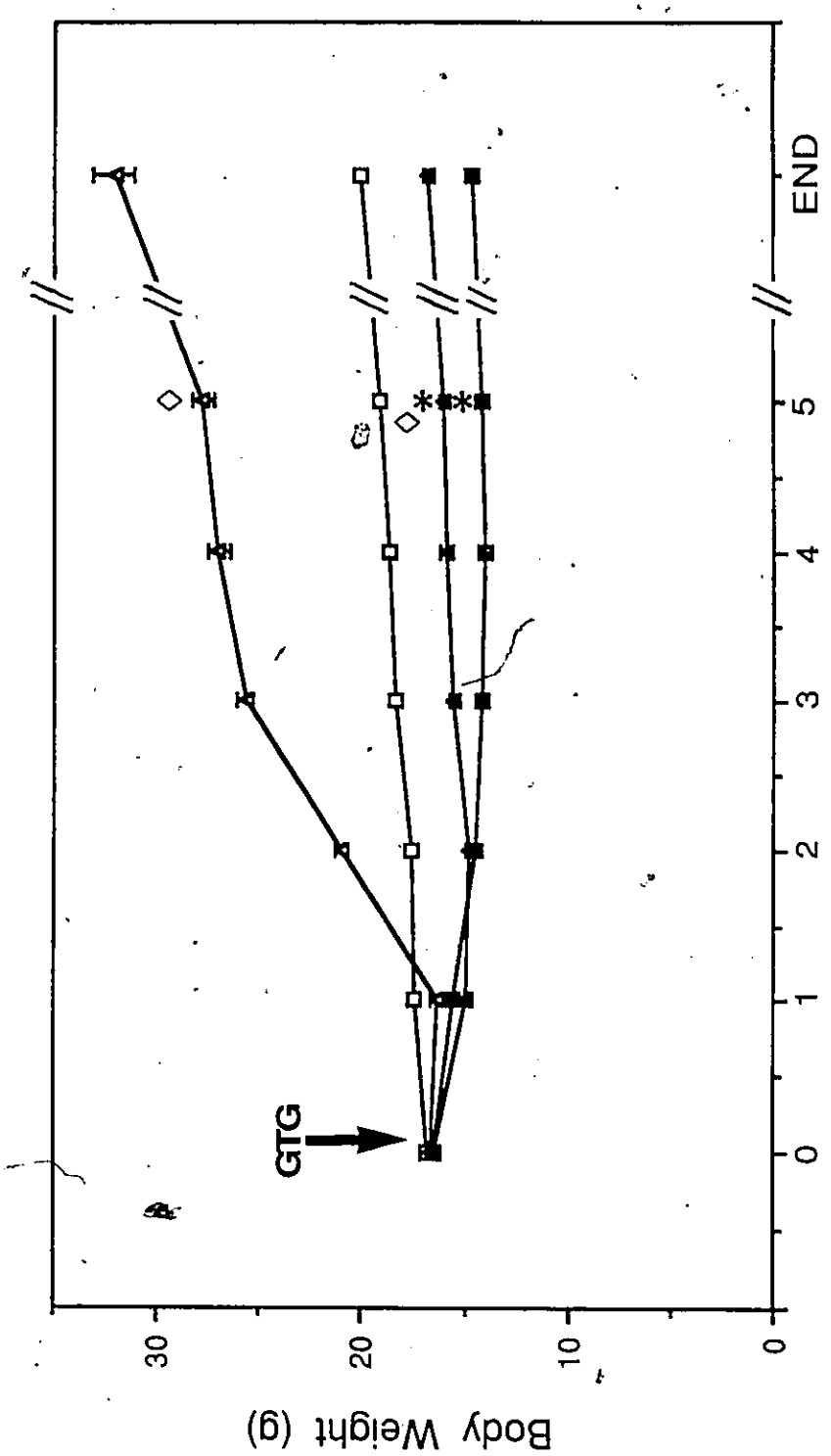


FIGURE 1b

body weight was increased by cafeteria-feeding and decreased by restricted-feeding.

For all diet groups, the weight of gonadal WAT was increased by the GTG-induced lesion (table 1). Cafeteria-fed mice of both types accumulated more WAT than their chow-fed counterparts. Cafeteria-fed GTG-obese mice had over four times more WAT than lean cafeteria-fed animals; however, the relative increase in WAT was approximately the same for both cafeteria-fed groups (2.5 times more WAT than their respective chow-fed controls). The weight of WAT was reduced by restricted feeding in both lean and GTG-lesioned mice. Nonetheless, GTG-lesioned mice had nearly four times as much WAT as lean mice, though both groups received identical amounts of food.

2. FOOD INTAKE

For each intake study, GTG-obese mice receiving chow or cafeteria diet were hyperphagic with respect to similarly-fed lean mice (table 2). Cafeteria-fed GTG-obese mice consumed more calories than chow-fed GTG-obese mice during the first intake period only. There was no significant difference in caloric intake between lean mice fed chow and cafeteria diets for any intake period. No effect of duration of feeding upon caloric consumption was seen for either chow-fed group or for lean mice fed cafeteria diet. That is to say, there was no change in daily caloric intake

TABLE 1: TISSUE WEIGHTS AND BAT PROTEIN OF LEAN AND GTG-OBESE MICE FED CHOW, RESTRICTED AND CAFETERIA DIETS

Animals were sacrificed 6-8 weeks after the indicated groups received the GTG lesion. Values represent means ± SE for 34 or more mice.

SYMBOLS:

* Significant effect of diet, comparing the same type of mouse.

◇ Significant effect of GTG lesion, comparing mice eating the same diet

	LEAN MICE			GTG-OBESE MICE		
	CHOW-FED	FOOD RESTRICTED	CAFETERIA-FED	CHOW-FED	FOOD RESTRICTED	CAFETERIA-FED
GONADAL WAT (mg)	336 ± 32.1	* 80 ± 8.6	* 856 ± 89.2	◇ 1695 ± 83.9	*◇ 314 ± 18.8	*◇ 4121 ± 136.2
BAT						
WET WEIGHT (mg)	236 ± 5.8	248 ± 7.9	* 305 ± 13.3	◇ 545 ± 18.8	*◇ 289 ± 6.4	*◇ 851 ± 31.7
PROTEIN (mg)	10.2 ± 0.28	* 8.8 ± 0.21	* 11.6 ± 0.34	9.6 ± 0.20	* 8.9 ± 0.24	* 11.9 ± 0.64

TABLE 1

TABLE 2: ENERGY INTAKES AND DIET COMPOSITION FOR LEAN AND GTG-OBESE MICE FED CHOW AND CAFETERIA DIET

24-hour food intake measurements were performed twice for each of the 3 cafeteria diets, and simultaneously twice each week for chow-fed groups. Values represent means ± SE for 12 or more cages of chow-fed and 10 or more cages of cafeteria-fed animals. The results were divided by the number of mice per cage (usually 4) to yield the average intake per mouse. Data were analyzed by 3-way repeated measures ANOVA and by Dunnett's and Simple Means post hoc tests. Cafeteria menus and diet composition are described in Appendix B. For reference, the composition of the chow diet was 23.5 % protein, 52.3 % carbohydrate, and 4.5 % fat.

SYMBOLS:

CHO = carbohydrate

* Significant effect of cafeteria diet upon caloric intake, comparing the same type of mouse

◇ Significant effect of GTG lesion, comparing mice eating the same diet

◆ Significant effect of duration of feeding for animals in the group indicated

	WEEK 1		WEEK 2		WEEK 3	
	INTAKE 1	INTAKE 2	INTAKE 3	INTAKE 4	INTAKE 5	INTAKE 6
LEAN MICE						
CHOW-FED						
kcal/mouse	11.0 ± 0.4	11.2 ± 0.4	10.7 ± 0.3	11.6 ± 0.4	11.2 ± 0.4	11.6 ± 0.7
CAFETERIA-FED						
kcal/mouse	10.0 ± 0.5	10.4 ± 0.5	9.5 ± 0.4	10.1 ± 0.3	11.0 ± 0.8	10.9 ± 0.4
% protein	10.7 ± 0.5	11.2 ± 0.4	10.6 ± 0.5	11.3 ± 0.2	11.4 ± 0.4	10.2 ± 0.4 ◆
% CHO	32.8 ± 0.9	33.2 ± 0.7	35.4 ± 1.1	32.8 ± 1.2	32.6 ± 1.2	34.4 ± 0.9
% fat	56.5 ± 1.0	55.6 ± 0.6	53.9 ± 1.3	56.0 ± 1.2	56.5 ± 0.9	55.4 ± 1.0
GTG-OBESE MICE						
CHOW-FED						
kcal/mouse	14.2 ± 0.4 *◇	17.7 ± 0.5◇	16.8 ± 0.6◇	15.2 ± 0.5◇	15.8 ± 0.8◇	14.5 ± 0.8◇
CAFETERIA-FED						
kcal/mouse	21.8 ± 1.5◇	16.3 ± 1.0◇	16.4 ± 0.8◇	16.5 ± 1.3◇	16.1 ± 1.0◇	15.8 ± 1.2◇◆
% protein	11.0 ± 0.3	12.1 ± 0.3	11.2 ± 0.6	12.0 ± 0.4	12.0 ± 0.5	10.4 ± 0.7 ◆
% CHO	30.6 ± 1.9	29.5 ± 1.1	28.4 ± 1.4◇	27.0 ± 1.6◇	28.4 ± 1.4◇	24.1 ± 0.3◇◆
% fat	58.4 ± 1.7	58.4 ± 0.9	60.4 ± 1.5◇	61.0 ± 1.5◇	59.6 ± 1.0◇	65.7 ± 0.6◇◆

during the period of study. An overall significant effect of feeding duration was observed for cafeteria-fed GTG-obese mice, but this was due to the larger intake of these animals during the first study period only. Subsequent caloric intake was relatively uniform in this group.

Diets chosen by both cafeteria groups were higher in fat and lower in protein and carbohydrate than the standard rat chow offered to chow-fed mice (data not analyzed statistically). The proportion of nutrients chosen by cafeteria-fed lean mice remained constant for the duration of the experiment. There was no significant difference between lean and GTG-obese mice in the percentage of protein consumed. However, a significant change with duration of feeding occurred in the fat and carbohydrate content of the diet chosen by GTG-obese mice; this change resulted in a higher fat consumption and a lower carbohydrate consumption than that of lean mice for the last four intake studies. The significant effect of feeding duration on protein intake of GTG-obese mice results from the high value observed in the second intake period (12.1%, table 2).

3. TEMPERATURE MEASUREMENTS

a) CIRCADIAN VARIATION:

Chow-fed lean and GTG-obese mice showed similar rhythms in rectal temperature, with highest levels occurring during the dark phase, and lowest levels occurring during the late

part of the light phase (figures 2a and 2b). Chow-fed GTG-obese mice had lower body temperatures than chow-fed lean mice at all time points except 1600h. The higher temperature of chow-fed GTG-obese mice at this point was due to the earlier start of their rewarming; the rate of rewarming was slower, however, and by 2000h the temperature of GTG chow-fed mice was again lower than that of chow-fed lean mice.

Restricted feeding of lean and GTG-obese mice resulted in large differences in rectal temperature in comparison with chow-fed controls. Food-restricted lean mice maintained low body temperatures for the first part of each day, between midnight and noon. They began to rewarm at 1000h, reaching their maximum temperature just after feeding, and remaining warm for the first six hours of the dark period. A similar pattern occurred in food-restricted GTG-lesioned mice, although rewarming began earlier and proceeded more slowly than for lean animals. Food-restricted GTG mice did not maintain their feeding-time temperature, but began to cool when the lights went out. Temperatures of food-restricted GTG-lesioned mice were lower than for lean mice at all times except the feeding time.

In lean mice, cafeteria feeding had no effect upon circadian variation in rectal temperature (figure 2a). Cafeteria-feeding of GTG-obese mice resulted in higher rectal temperatures in the late morning and early afternoon

FIGURE 2a: CIRCADIAN VARIATION IN BODY TEMPERATURE OF LEAN AND GTG-OBESE MICE: EFFECT OF CAFETERIA DIET

Mice were studied 3-6 weeks after lesioning, after receiving cafeteria diet for at least two weeks. Cafeteria food was presented at 1500h each day. Lights were on between 0600h and 1800h. Values at each time point represent means ± SE for 8 or more lean, and 6 or more GTG-obese mice.

SYMBOLS:

-□- **LEAN CHOW-FED**

-■- **LEAN CAFETERIA-FED**

-△- **GTG CHOW-FED**

-▲- **GTG CAFETERIA-FED**

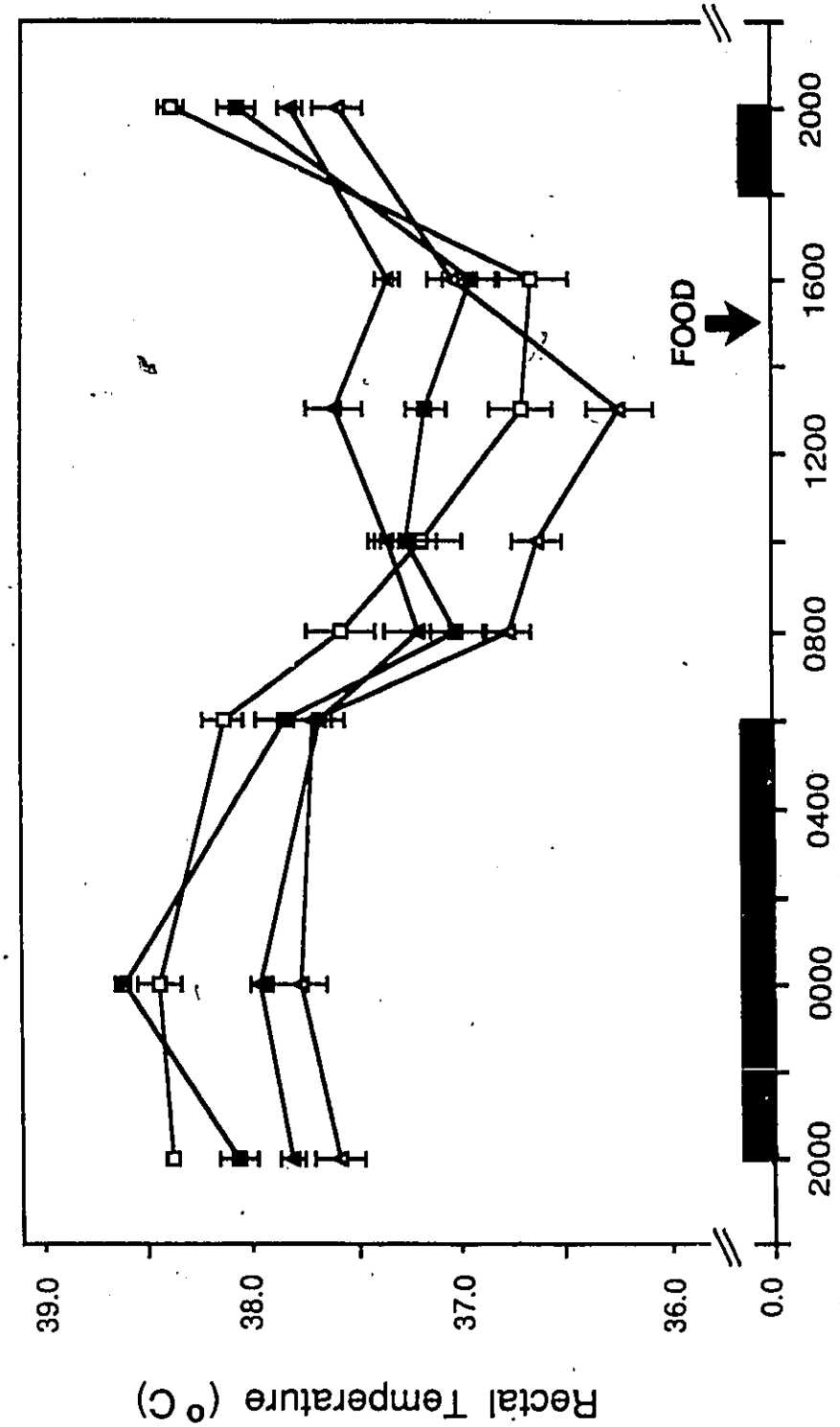

Rectal Temperature (°C)
39.0
38.0
37.0
36.0
0.0
2000
0000
0400
0800
1200
1600
2000
TIME OF DAY
FOOD

FIGURE 2a

FIGURE 2b: **CIRCADIAN VARIATION IN BODY TEMPERARURE OF LEAN AND GTG-OBESE MICE: EFFECT OF FOOD RESTRICTION**

Mice were studied 3-6 weeks after lesioning, after food restriction for the same period. Values at each time point represent means ± SE for 12 or more lean, and 14 or more GTG-obese mice. For further information, see legend to figure 2a.

SYMBOLS:

-□- LEAN CHOW-FED

-■- LEAN FOOD-RESTRICTED

-△- GTG CHOW-FED

-▲- GTG FOOD-RESTRICTED

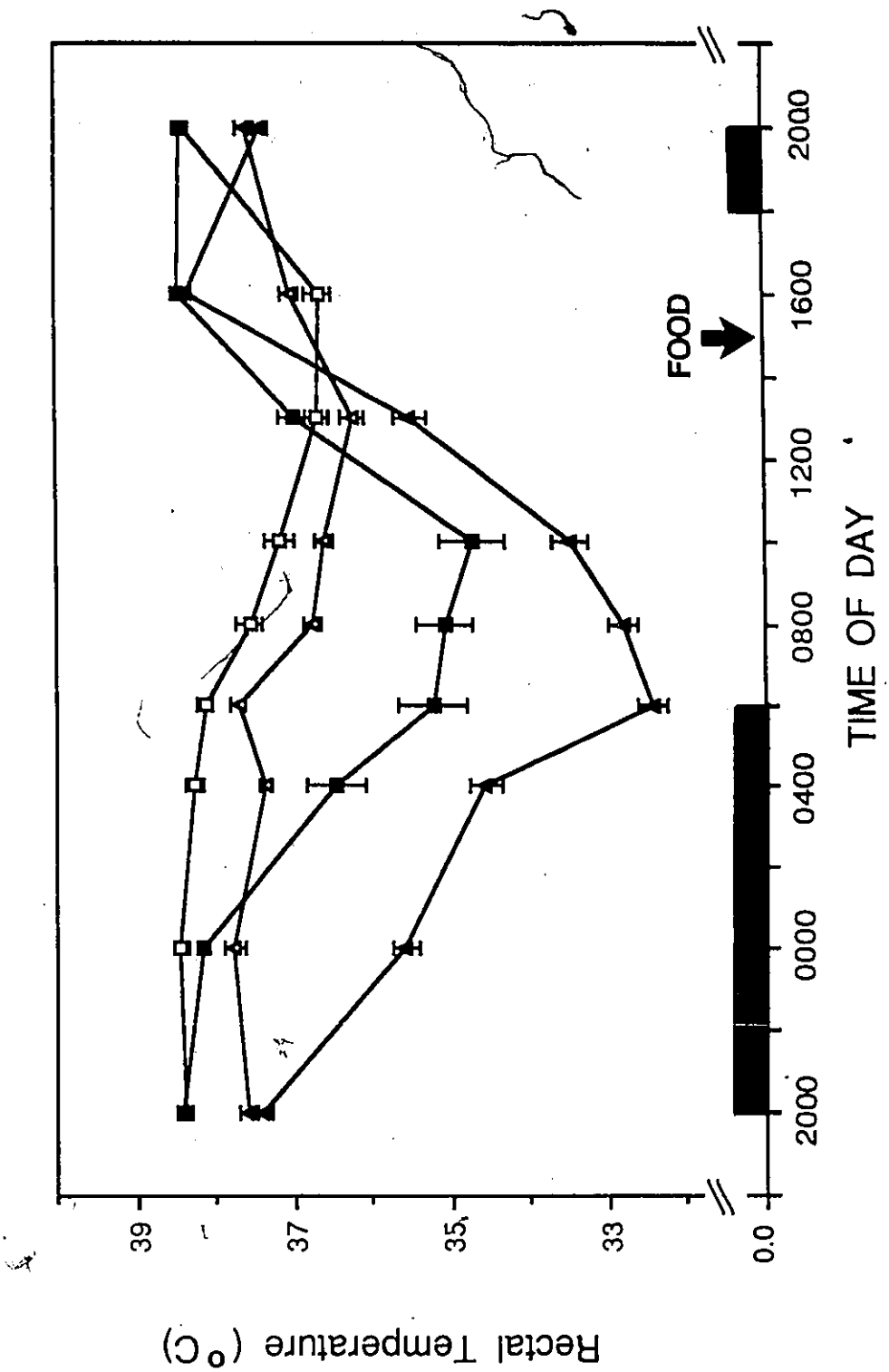

Rectal Temperature (°C)
39
37
35
33
0.0
2000
0000
0400
0800
1200
1600
2000
TIME OF DAY
FOOD

FIGURE 2b

(1100h, 1300h), compared with chow-fed GTG-obese animals. The two groups of cafeteria-fed mice appeared to differ at 0000h only, when the rectal temperature of lean mice exceeded that of GTG-obese mice.

b) RECTAL TEMPERATURE AT SACRIFICE

The circadian pattern in rectal temperatures of mice sacrificed at different time points agreed fairly well with the results presented in figures 2a and 2b (figure 3). The temperatures of food-restricted mice were again reduced in comparison with chow-fed animals, and cafeteria feeding again resulted in an increased temperature in GTG-obese mice in the late morning and early afternoon. However, the effect of obesity that was apparent in chow and food-restricted mice during measurement of circadian rhythm was not present at the time of sacrifice. As well, in lean mice, an increased body temperature was observed at 1500h. These results differed from those obtained by the initial measurement of circadian variation in rectal temperature.

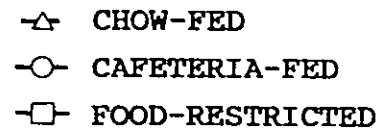
4. GROWTH OF BROWN ADIPOSE TISSUE

No effect of the GTG-induced lesion on total BAT protein was observed for mice in any diet group, although in all cases the wet weight of BAT was increased in GTG-lesioned animals (table 1). These results suggest that GTG-induced obesity promoted a marked lipid accumulation in BAT, rather than a change in its metabolic mass. Total BAT protein was

FIGURE 3: RECTAL TEMPERATURES OF CAFETERIA- AND RESTRICTED-FED LEAN AND GTG-OBESE MICE AT SACRIFICE

The times of day studied are: Cafeteria-fed mice, 0500h, 1100h, 1500h, 1900h, 2400h; food-restricted mice, 0500h, 0700h, 1100h, 1500h; chow-fed mice, 0500h, 0700h, 1100h, 1500h, 1900h, 2400h; Diets were presented to cafeteria and restricted-fed animals at 1500h daily. Animals in the 1500h groups were killed 10-15 minutes after the food was presented. Lights were on between 0600h and 1800h. Values represent means ± SE for 5 or more GTG cafeteria-fed animals, and 8 or more mice from all other groups.

SYMBOLS:



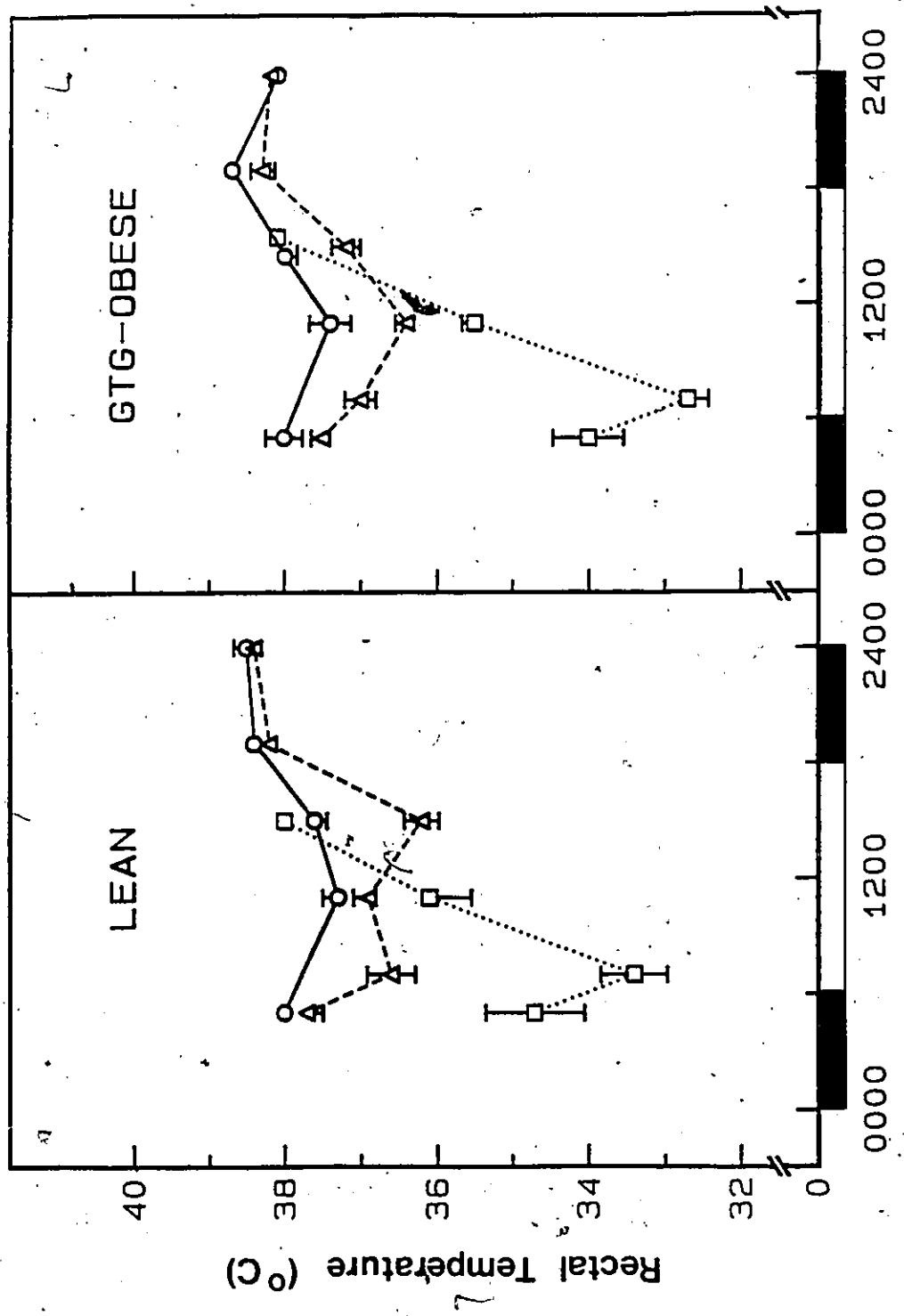

GTG-OBESE
LEAN
Rectal Temperature (°C)
40
38
36
34
32
0
0000
1200
2400
0000
1200
2400
TIME OF DAY

FIGURE 3

increased by cafeteria feeding in both lean and GTG-obese mice, reflecting a true growth of the tissue. The increase in BAT protein prompted by cafeteria-feeding was not appreciably greater in cafeteria-fed GTG-obese mice than in cafeteria-fed lean animals. Restricted feeding resulted in a large decrease in the wet weight of BAT in GTG-obese mice, reflecting, at least in part, a reduced lipid content in the tissue (table 1). In both lean and GTG-lesioned mice, a small but significant decrease in total BAT protein was promoted by food restriction.

5. THERMOGENIC ACTIVATION OF BAT MITOCHONDRIA

Cafeteria feeding increased the binding of GDP to isolated BAT mitochondria in both lean and GTG-obese mice, indicating thermogenic activation of the tissue (figures 4 and 5a). The two groups differed in the proportion of the day for which this activation was observed. In lean mice, GDP binding was increased at all measured time points excluding 1100h, while in GTG-lesioned animals binding was significantly increased by cafeteria-feeding during the afternoon and evening time points only (1500h, 1900h, 2400h). Compared to lean cafeteria-fed mice, GDP-binding was significantly lower for GTG cafeteria-fed animals, except at 1100h, when neither group were thermogenically active, and at 1500h, the feeding time. At 1500h, GDP-binding in cafeteria-fed GTG-obese mice approximated the

FIGURE 4: CIRCADIAN VARIATION IN BAT MITOCHONDRIAL GDP-BINDING OF CAFETERIA-FED AND FOOD-RESTRICTED LEAN AND GTG-OBESE MICE

The times of day studied are: cafeteria-fed mice, 0500h, 1100h, 1500h, 1900h, 2400h; food-restricted mice, 0500h, 0700h, 1100h, 1500h; chow-fed mice, 0500h, 0700h, 1100h, 1500h, 1900h, 2400h; Diets were presented to cafeteria- and restricted-fed animals at 1500h daily. Animals in the 1500h groups were killed 10-15 minutes after the food was presented. Lights were on between 0600h and 1800h. Values represent means ± SE for 8 or more lean chow-, lean cafeteria-, and GTG chow-fed animals, 5 or more GTG cafeteria-fed animals, and 4 or more food-restricted animals.

SYMBOLS:

-△- CHOW-FED

-○- CAFETERIA-FED

-□- FOOD-RESTRICTED

* Significant effect of diet, comparing the same type of mouse

◆ Significant circadian variation in BAT mitochondrial GDP-binding for the group indicated

Effects of the GTG lesion are shown in figures 5a and 5b

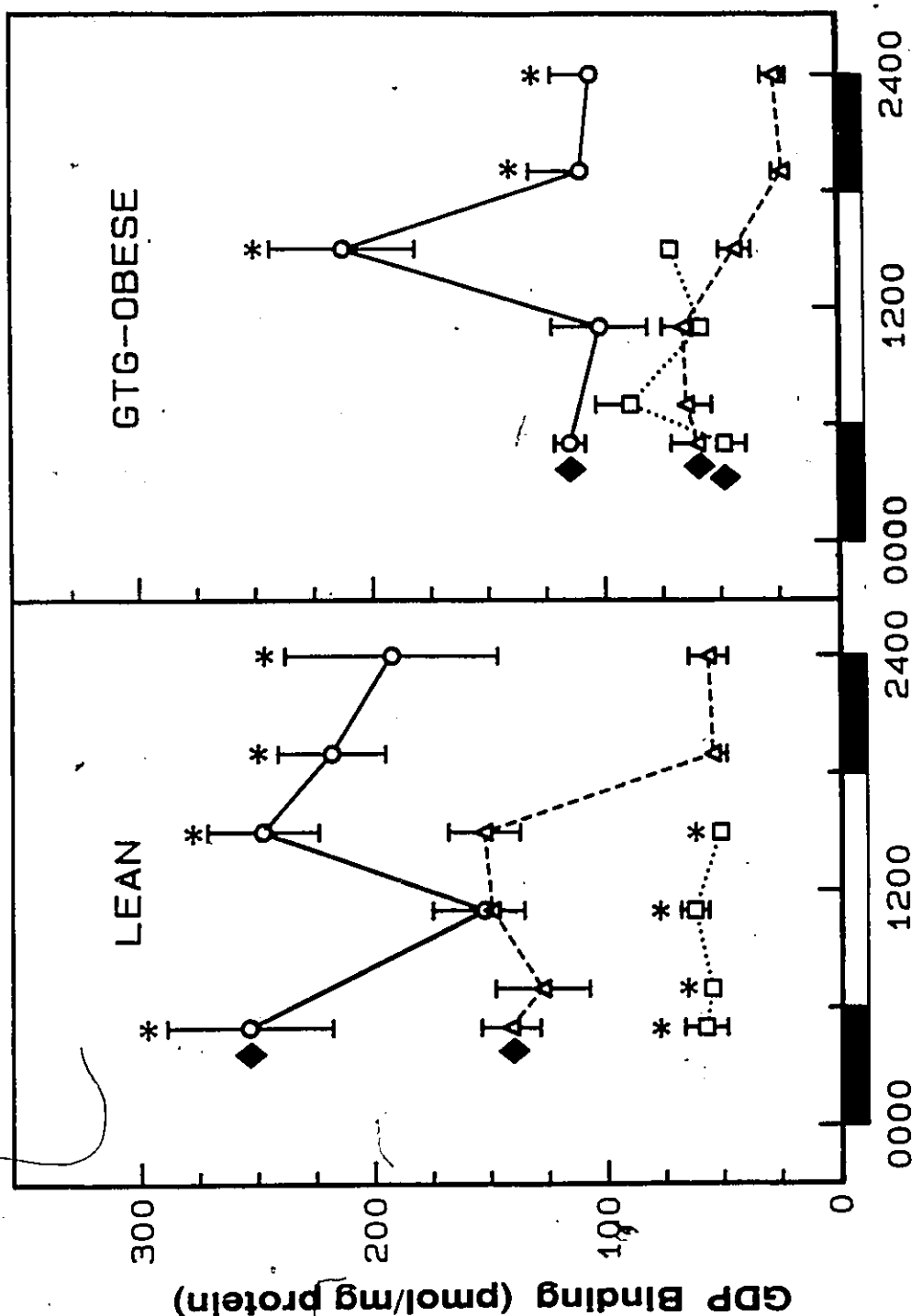

GTG-OBESE
LEAN
GDP Binding (pmol/mg protein)
300
200
100
0
0000
1200
2400 0000
1200
2400
TIME OF DAY

FIGURE 4

FIGURE 5a: MITOCHONDRIAL GDP-BINDING OF LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW AND CAFETERIA-FED MICE

Data from figure 4 have been replotted to allow an easier assessment of the effect of GTG-lesion on GDP-binding of mice eating the same diet. Chow- and cafeteria-fed mice were killed at the times shown. Food was presented to cafeteria-fed mice at 1500h. Lights were on between 0600h and 1800h. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for cafeteria-fed animals. Values represent means ± SE for 8 or more lean chow-, lean cafeteria- and GTG chow-fed animals, and 5 or more GTG cafeteria-fed animals.

* Significant effect of diet, comparing the same type of mouse

◇ Significant effect of GTG-treatment, comparing mice eating the same diet

FIGURE 5b: MITOCHONDRIAL GDP-BINDING OF LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW-FED AND FOOD-RESTRICTED ANIMALS

Data have been replotted from figure 4. Rations were presented to food-restricted mice at 1500h. Note that the time points measured for these animals are different from those in the above figure. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for food-restricted mice. Values represent means ± SE for 8 or more lean and GTG-obese chow-fed mice, and 4 or more lean and GTG obese food-restricted mice. For further information see figure 5a.

FIGURE 5a

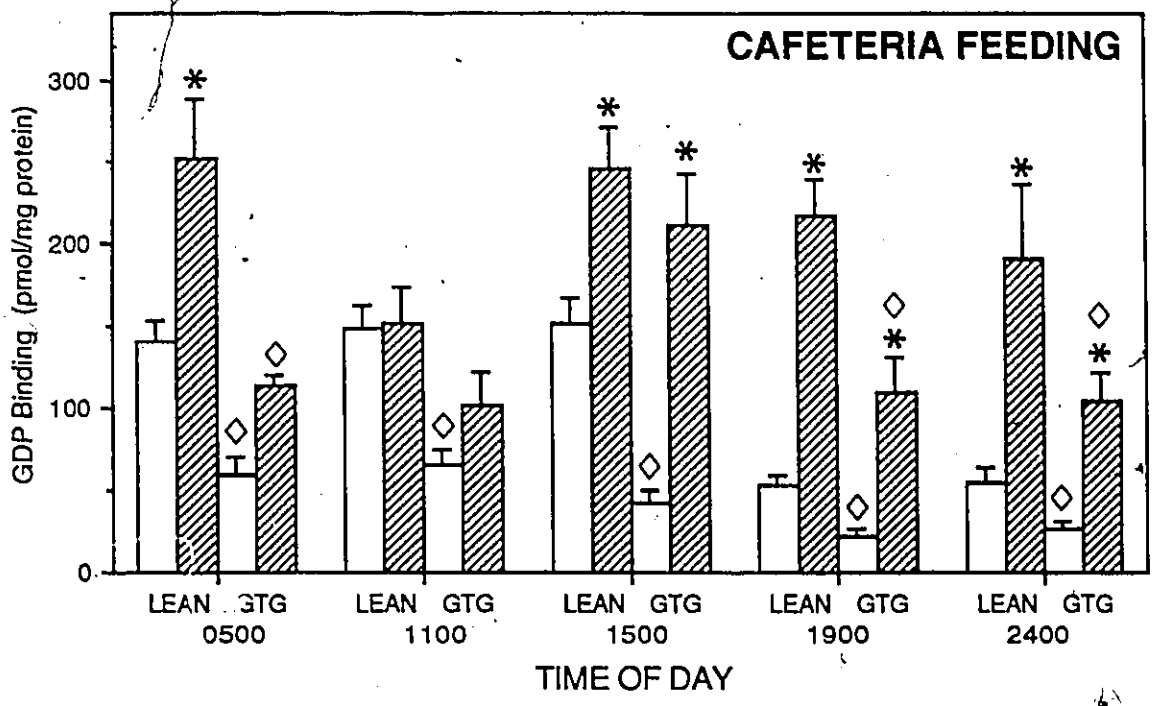
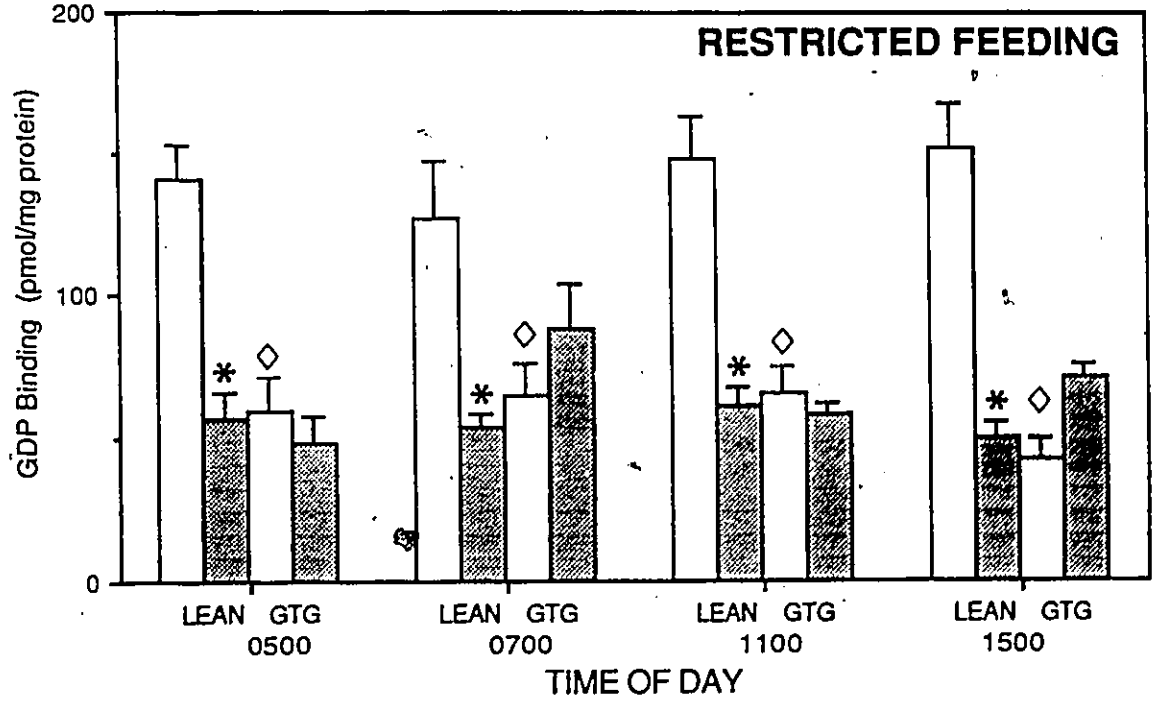


FIGURE 5b



highest levels observed for lean cafeteria-fed mice at any time of day.

Food-restriction resulted in suppression of GDP-binding in lean mice at all measured times (figures 4 and 5b). There was no effect of food restriction on GDP-binding of mitochondria from GTG-obese mice, except at the feeding time, when a small but significant increase was observed. In chow-fed animals, GDP-binding was reduced in GTG-obese mice at all times of day, suggesting a lower basal thermogenic activation of BAT in this group.

A circadian variation in GDP binding was apparent in all experimental groups, except for food-restricted lean mice (figure 4). However, the daily pattern of binding was markedly altered by diet treatment. In chow-fed groups, the daily variation in GDP binding was similar for lean and GTG-obese mice. The highest levels of binding were observed in the early morning and the light phase, with much lower levels occurring after lights out.

The pattern of GDP binding observed for cafeteria-fed mice bore little resemblance to that of chow-fed animals and a marked difference existed between cafeteria-fed lean and GTG-obese mice. Lean mice had a high level of binding which fell only at the 1100h time point. In comparison, GTG-obese animals maintained a much lower level of binding which rose only at the feeding time. The variation in binding seen in chow-fed lean mice was completely abolished by food-

restriction. In food-restricted GTG-obese mice, the statistically significant effect of time of day resulted from an increase in binding after lights on. The higher binding was maintained at a constant level for the remaining time points.

6. ACTIVITY OF BAT THYROXINE 5'-DEIODINASE

A significant circadian variation was observed in specific BAT 5'-deiodinase activity of all experimental groups, excepting food-restricted lean mice (figure 6). The circadian pattern in enzyme activity was similar for all treatments, with nadirs occurring at 1100h, and peaks between 1500h and 1900h. No effect of diet treatment was observed in lean or obese mice, except at 0500h, when BAT 5'-DII activity was greatly increased in cafeteria-fed GTG-obese mice (figure 7a). Similarly, the GTG-induced lesion had little effect on enzyme activity, except at 0500h (cafeteria-fed GTG-obese higher than cafeteria-fed lean), and 1500h (food-restricted GTG-obese higher than food-restricted lean) (figure 7b). Results for total 5'DII activity showed a similar pattern of circadian variation (figure 8) and similar treatment effects (figures 9a and 9b) as those described for the specific activity of this enzyme, except that there was no effect of GTG-induced obesity on total deiodinase activity of food-restricted mice.

FIGURE 6: CIRCADIAN VARIATION IN SPECIFIC ACTIVITY OF BAT T_4 5'-DEIODINASE IN CAFETERIA-FED AND FOOD-RESTRICTED LEAN AND GTG-OBESE MICE

The times of day studied are: cafeteria-fed mice, 0500h, 1100h, 1500h, 1900h, 2400h; food-restricted mice, 0500h, 0700h, 1100h, 1500h; chow-fed mice, 0500h, 0700h, 1100h, 1500h, 1900h, 2400h; Diets were presented to cafeteria- and restricted-fed animals at 1500h daily. Animals in the 1500h groups were killed 10-15 minutes after the food was presented. Lights were on between 0600h and 1800h. Values represent means for 5 or more GTG cafeteria-fed animals and 8 or more animals in all other groups.

SYMBOLS:

-△- **CHOW-FED**

-○- **CAFETERIA-FED**

-□- **FOOD-RESTRICTED**

* Significant effect of diet, comparing mice of the same type

◆ Significant circadian variation in specific activity of BAT T_4 5'-deiodinase activity for the group indicated

Effects of the GTG lesion are shown in figures 7a and 7b

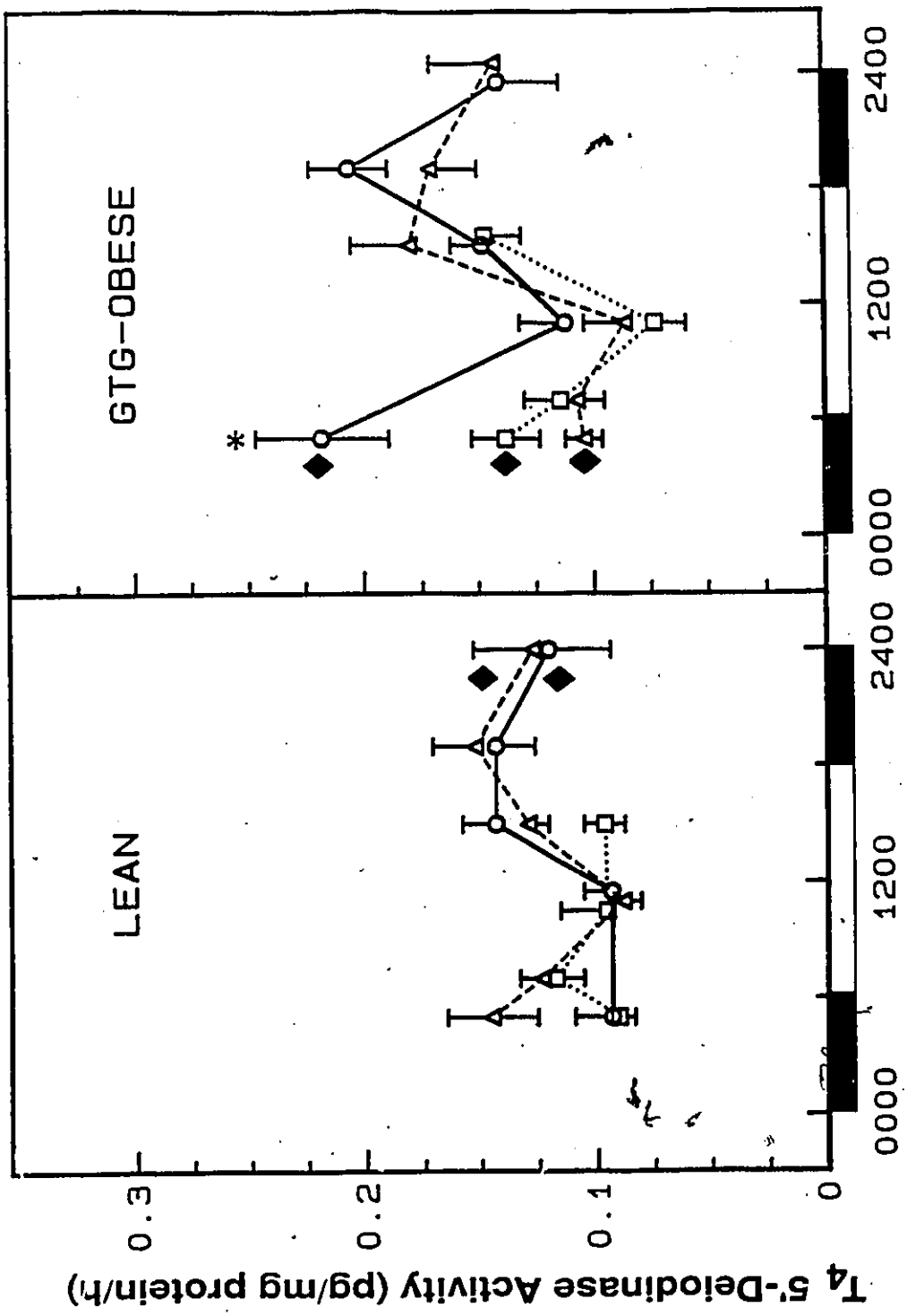

GTG-OBESE
LEAN
*
0.3
0.2
0.1
0
0000
1200
2400 0000
1200
2400
TIME OF DAY
T4 5'-Deiodinase Activity (pg/mg protein/h)

FIGURE 6

FIGURE 7a: SPECIFIC ACTIVITY OF BAT T_4 5'-DEIODINASE IN LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW AND CAFETERIA-FED MICE

Data from figure 6 have been replotted to allow an easier assessment of the effect of GTG-lesion on specific activity of BAT 5'DII of mice eating the same diet. Chow- and cafeteria-fed mice were killed at the times shown. Food was presented to cafeteria-fed mice at 1500h. Lights were on between 0600h and 1800h. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for cafeteria-fed animals. Values represent means ± SE for 8 or more lean chow-, lean cafeteria- and GTG chow-fed animals, and 5 or more GTG cafeteria-fed animals.

* Significant effect of diet, comparing the same type of mouse

◇ Significant effect of GTG-treatment, comparing mice eating the same diet

FIGURE 7b: SPECIFIC ACTIVITY OF BAT T_4 5'-DEIODINASE IN LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW-FED AND FOOD-RESTRICTED ANIMALS

Data have been replotted from figure 6. Rations were presented to food-restricted mice at 1500h. Note that the time points measured for these animals are different from those in the above figure. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for food-restricted mice. Values represent means ± SE for 8 or more mice. For further information see figure 7a.

FIGURE 7a

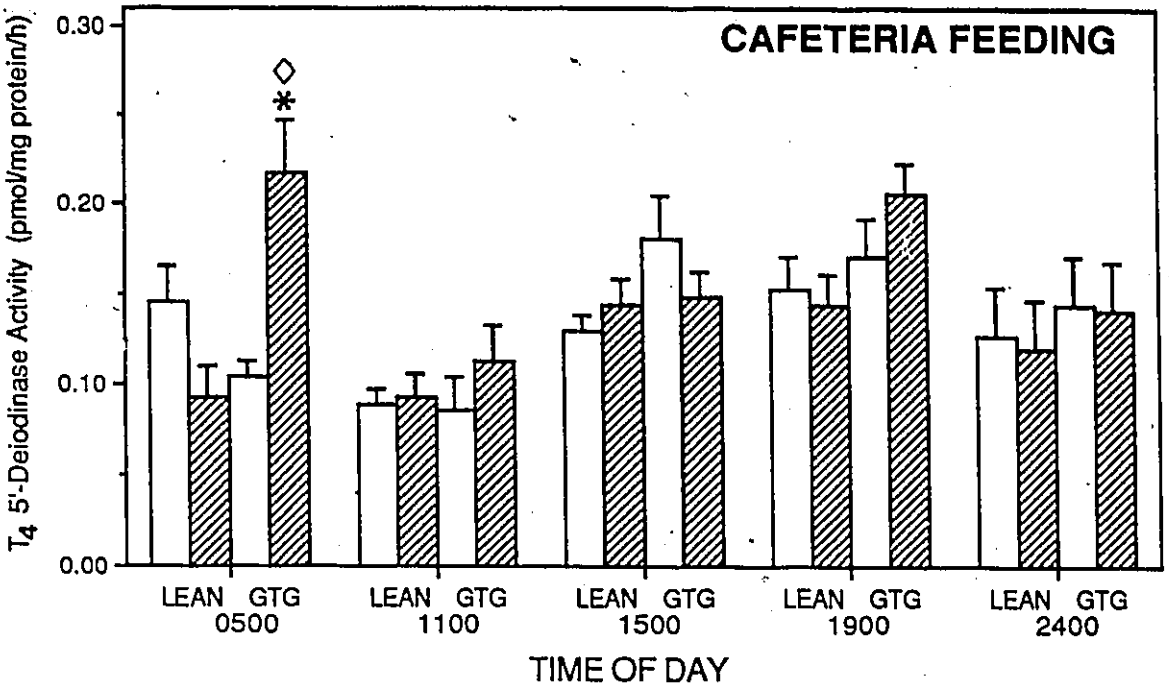


FIGURE 7b

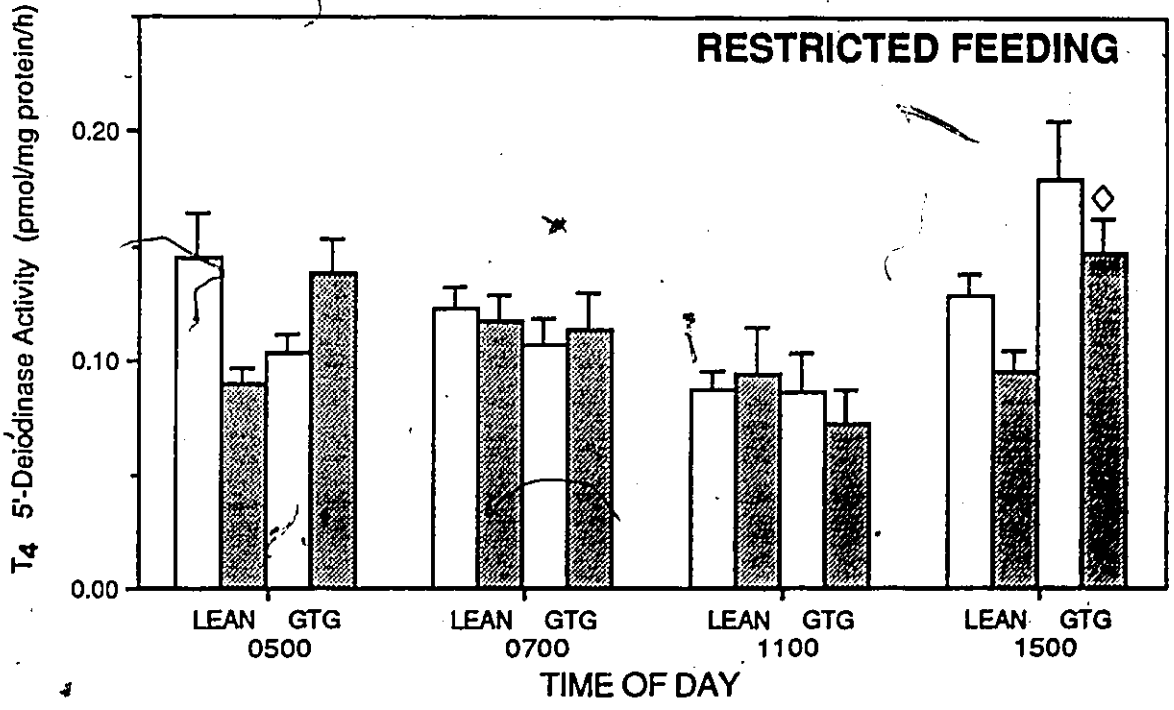


FIGURE 8: CIRCADIAN VARIATION IN TOTAL ACTIVITY OF BAT T_4 5'-DEIODINASE IN CAFETERIA-FED AND FOOD-RESTRICTED LEAN AND GTG-OBESE MICE

The times of day studied are: cafeteria-fed mice, 0500h, 1100h, 1500h, 1900h, 2400h; food-restricted mice, 0500h, 0700h, 1100h, 1500h; chow-fed mice, 0500h, 0700h, 1100h, 1500h, 1900h, 2400h; Diets were presented to cafeteria- and restricted-fed animals at 1500h daily. Animals in the 1500h groups were killed 10-15 minutes after the food was presented. Lights were on between 0600h and 1800h. Values represent means ± SE for 5 or more GTG cafeteria-fed animals and 8 or more animals in all other groups.

SYMBOLS:

△ **CHOW-FED**

○ **CAFETERIA-FED**

□ **FOOD-RESTRICTED**

* Significant effect of diet, comparing the same type of mouse

◆ Significant circadian variation in total activity of BAT T_4 5-deiodinase for the group indicated

Effects of GTG-lesion are shown in figures 9a and 9b

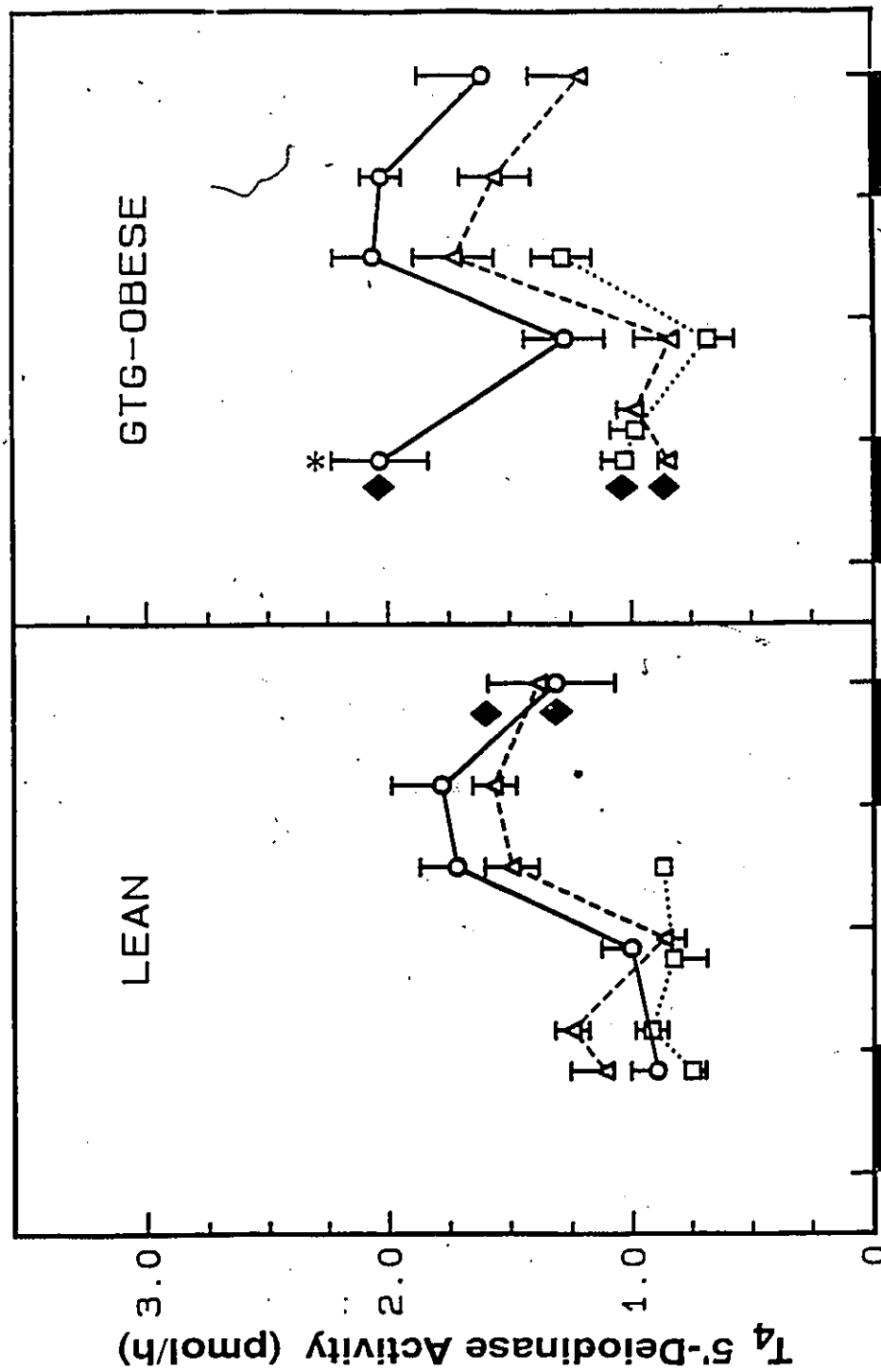

LEAN
GTG-OBESE
T_4 5'-Deiodinase Activity (pmol/h)
3.0
2.0
1.0
0
0000
1200
2400 0000
1200
2400
TIME OF DAY

FIGURE 8

FIGURE 9a: **TOTAL ACTIVITY OF BAT T_4 5'-DEIODINASE IN LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW AND CAFETERIA-FED MICE**

Data from figure 8 have been replotted to allow an easier assessment of the effect of GTG-lesion on total activity of BAT 5'DII of mice eating the same diet. Chow- and cafeteria-fed mice were killed at the times shown. Food was presented to cafeteria-fed mice at 1500h. Lights were on between 0600h and 1800h. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for cafeteria-fed animals. Values represent means ± SE for 8 or more lean chow-, lean cafeteria- and GTG chow-fed animals, and 5 or more GTG cafeteria-fed animals.

* Significant effect of diet, comparing the same type of mouse

◇ Significant effect of GTG-treatment, comparing mice eating the same diet

FIGURE 9b: **TOTAL ACTIVITY OF BAT T_4 5'-DEIODINASE IN LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW-FED AND FOOD-RESTRICTED ANIMALS**

Data have been replotted from figure 8. Rations were presented to food-restricted mice at 1500h. Note that the time points measured for these animals are different from those in the above figure. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for food-restricted mice. Values represent means ± SE for 8 or more mice. For further information see figure 7a.

FIGURE 9a

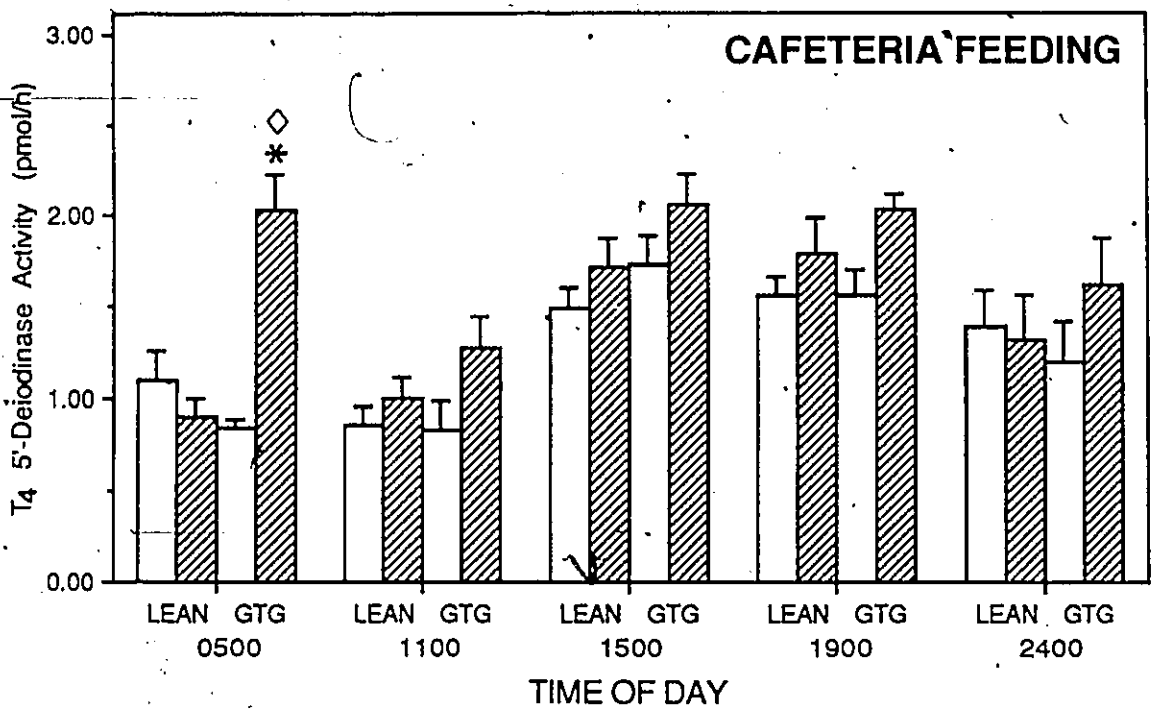
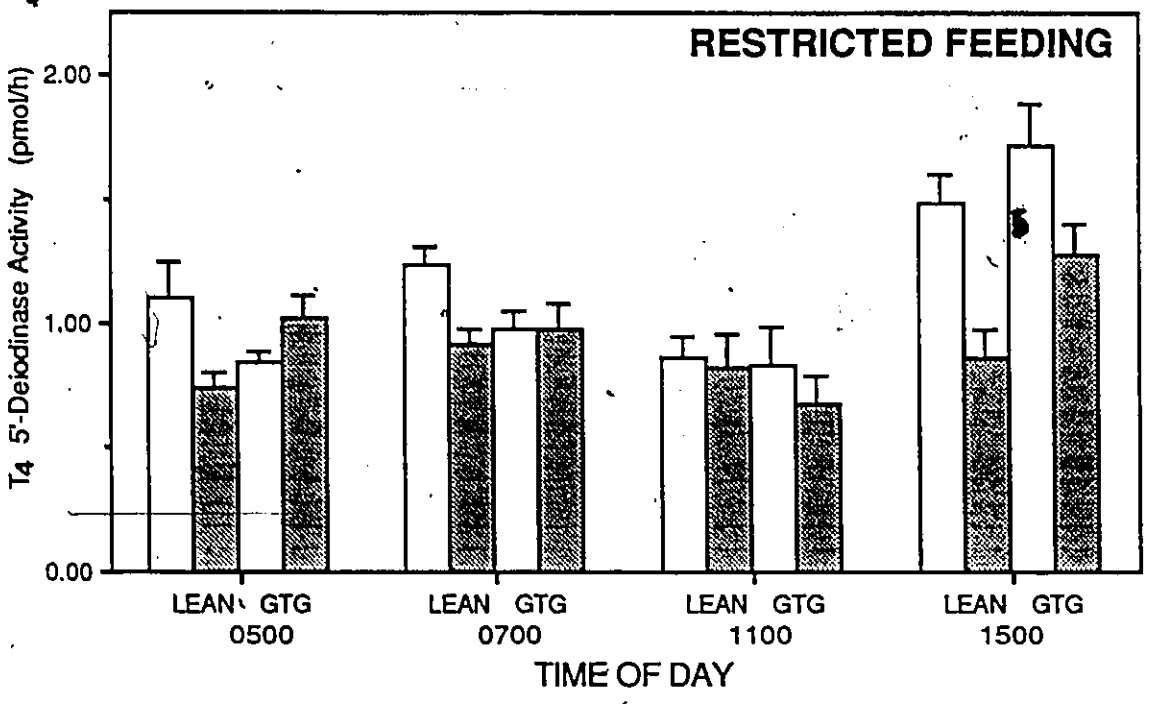


FIGURE 9b



7. THYROID HORMONES

A significant circadian rhythm was observed in serum T_3 concentration in all experimental groups (figure 10). Serum T_3 reached maximum levels in the morning hours and minimum levels in the early evening. Diet and obesity had little effect on the pattern of variation in this hormone. Serum T_3 levels were increased by cafeteria feeding and decreased by food-restriction in lean and GTG-obese animals at most time points (figures 11a and 11b). In food-restricted mice, GTG-induced obesity had no effect on serum T_3. Chow-fed GTG-obese mice had higher T_3 levels than their controls at 0500h and 1500h, and levels in cafeteria-fed GTG-obese mice exceeded those in cafeteria-fed lean mice in the early morning and throughout the light phase.

The circadian rhythm observed in serum T_4 concentration was similar to that of serum T_3 (figure 12). GTG-induced obesity and diet treatments had little effect on serum T_4 levels except at isolated time points (figures 13a and 13b). In GTG-obese animals there was a general trend toward increased T_4 with cafeteria or restricted diets, though only for a small part of the day. An effect of obesity was noted at 0500h only, when T_4 was significantly lower in GTG-obese cafeteria-fed animals.

FIGURE 10: CIRCADIAN VARIATION IN SERUM T_3 CONCENTRATION OF CAFETERIA-FED AND FOOD-RESTRICTED LEAN AND GTG-OBESE MICE

The times of day studied are: cafeteria-fed mice, 0500h, 1100h, 1500h, 1900h, 2400h; food-restricted mice, 0500h, 0700h, 1100h, 1500h; chow-fed mice, 0500h, 0700h, 1100h, 1500h, 1900h, 2400h; Diets were presented to cafeteria- and restricted-fed animals at 1500h daily. Animals in the 1500h groups were killed 10-15 minutes after the food was presented. Lights were on between 0600h and 1800h. Values represent means ± SE for 5 or more GTG cafeteria-fed animals and 8 or more animals in all other groups.

SYMBOLS:

-△- **CHOW-FED**

-○- **CAFETERIA-FED**

-□- **FOOD-RESTRICTED**

* Significant effect of diet, comparing the same type of mouse

◆ Significant circadian variation in serum T_3 concentration for the group indicated

Effects of the GTG lesion are shown in figures 11a and 11b

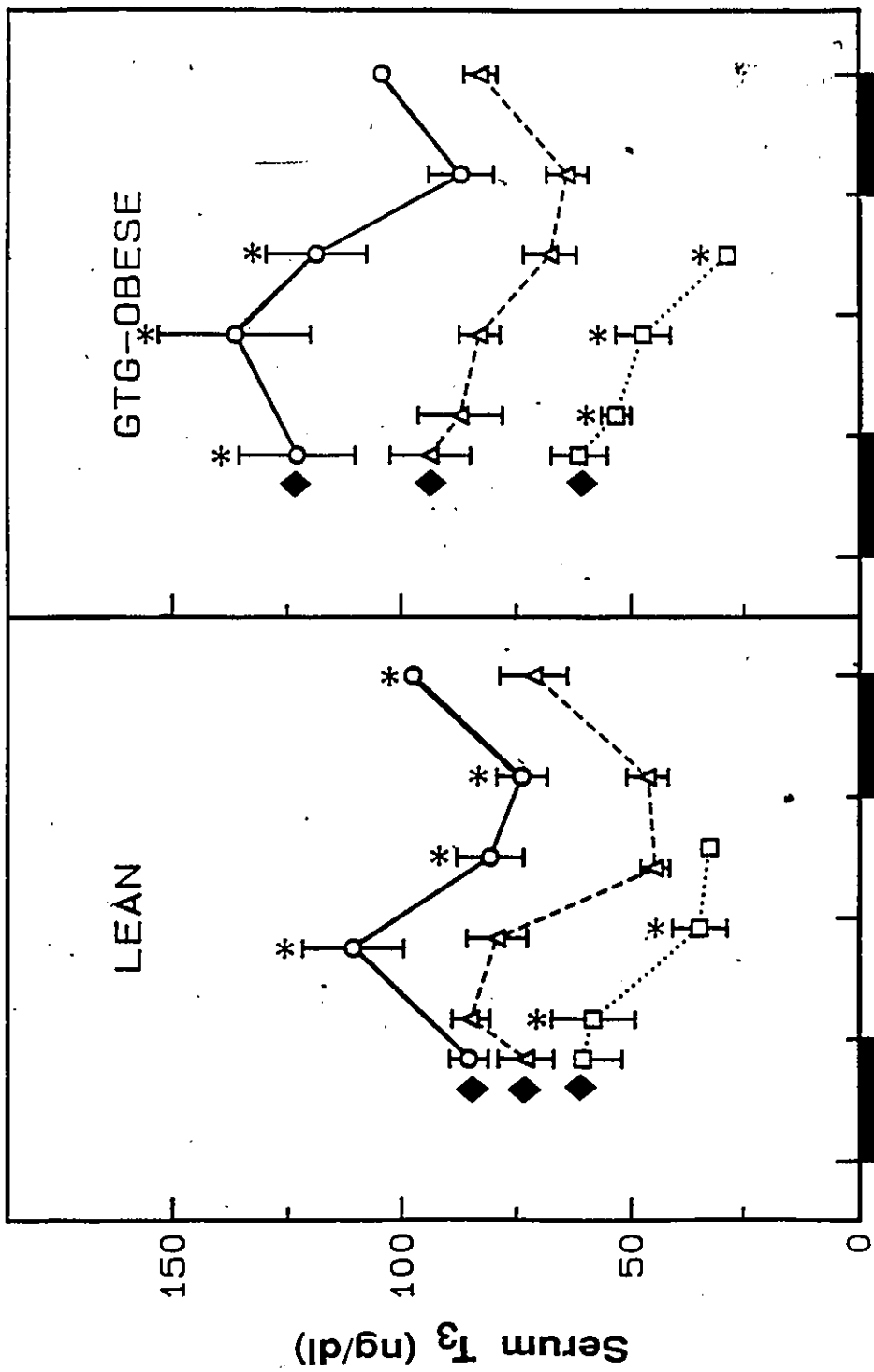

LEAN
GTG-OBESE
Serum T_3 (ng/dl)
150
100
50
0
0000
1200
2400 0000
1200
2400
TIME OF DAY

FIGURE 10

FIGURE 11a: SERUM T_3 CONCENTRATION OF LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW- AND CAFETERIA-FED MICE

Data from figure 10 have been replotted to allow an easier assessment of the effect of GTG-lesion on serum T_3 concentration in mice eating the same diet. Chow- and cafeteria-fed mice were killed at the times shown. Food was presented to cafeteria-fed mice at 1500h. Lights were on between 0600h and 1800h. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for cafeteria-fed animals. Values represent means ± SE for 8 or more lean chow-, lean cafeteroa and GTG chow-fed animals, and 5 or more GTG cafeteria-fed animals.

* Significant effect of diet, comparing the same type of mouse

◇ Significant effect of GTG-treatment, comparing mice eating the same diet

FIGURE 11b: SERUM T_3 CONCENTRATION OF LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW- AND RESTRICTED-FED ANIMALS

Data have been replotted from figure 10. Rations were presented to food-restricted mice at 1500h. Note that the time points measured for these animals are different from those in the above figure. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for food-restricted mice. Values represent means ± SE for 8 or more mice. For further information see figure 7a.

FIGURE 11a

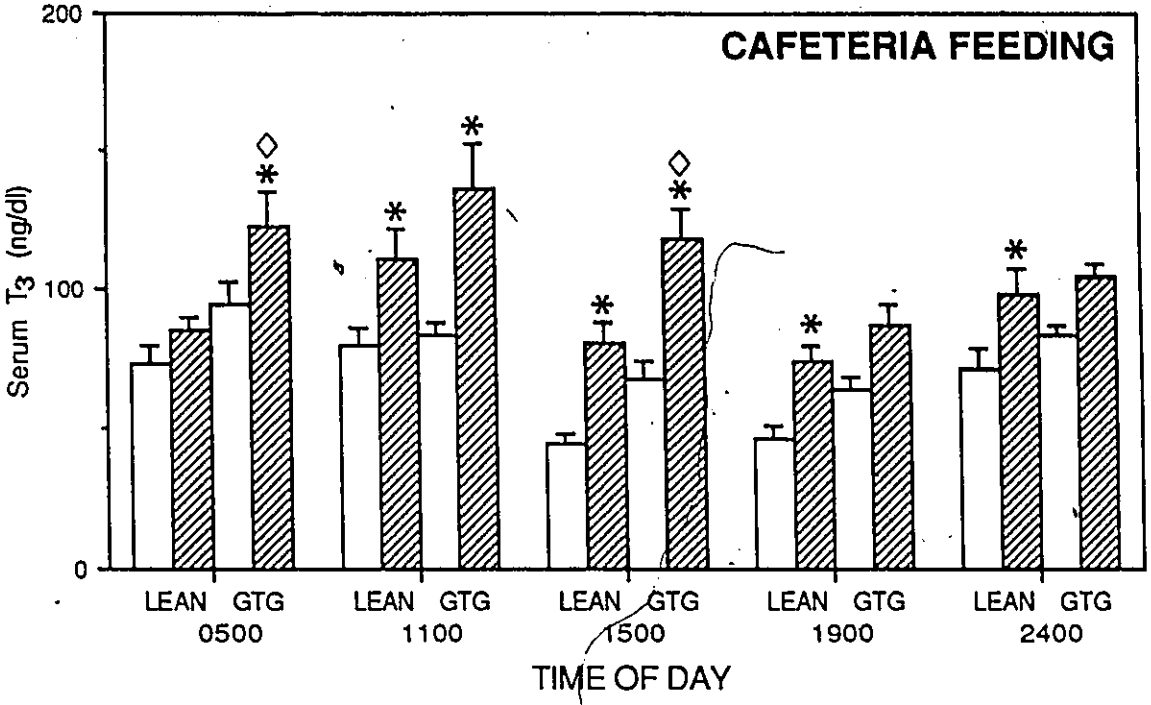


FIGURE 11b

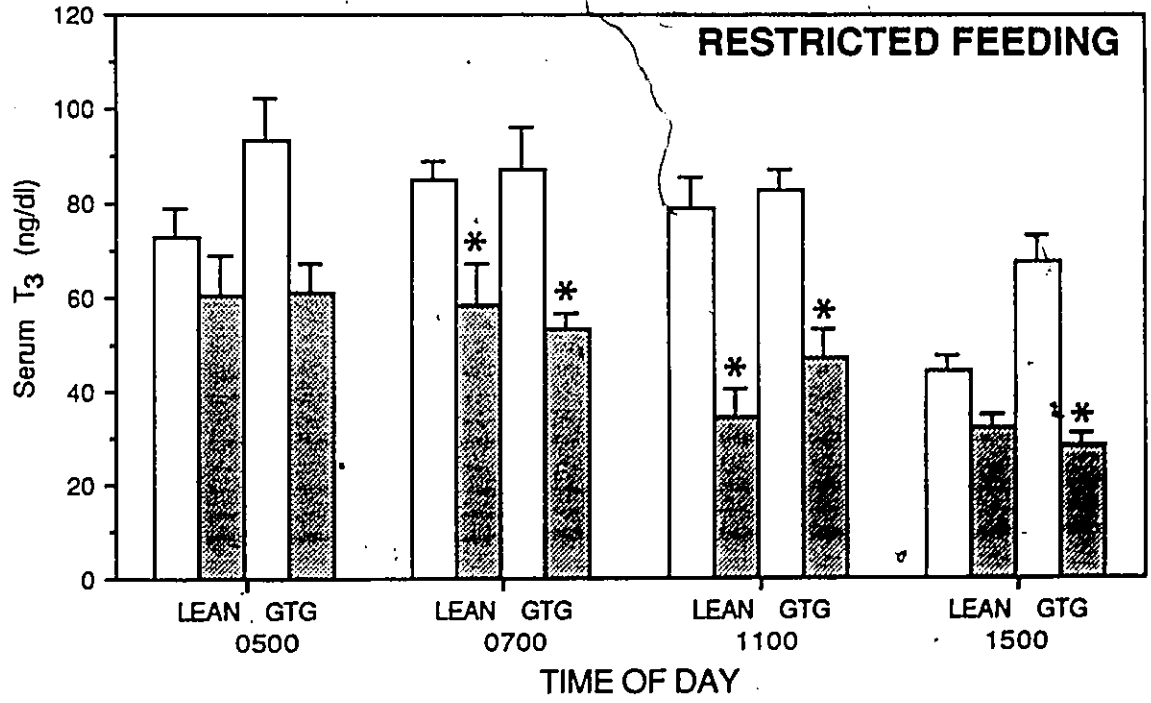


FIGURE 12: CIRCADIAN VARIATION IN SERUM T_4 CONCENTRATION OF CAFETERIA-FED AND FOOD-RESTRICTED LEAN AND GTG-OBESE MICE

The times of day studied are: cafeteria-fed mice, 0500h, 1100h, 1500h, 1900h, 2400h; food-restricted mice, 0500h, 0700h, 1100h, 1500h; chow-fed mice, 0500h, 0700h, 1100h, 1500h, 1900h, 2400h; Diets were presented to cafeteria- and restricted-fed animals at 1500h daily. Animals in the 1500h groups were killed 10-15 minutes after the food was presented. Lights were on between 0600h and 1800h. Values represent means ± SE for 5 or more GTG cafeteria-fed animals and 8 or more animals in all other groups.

SYMBOLS:

-△- **CHOW-FED**

-○- **CAFETERIA-FED**

-□- **FOOD-RESTRICTED**

* Significant effect of diet, comparing the same type of mouse

◆ Significant circadian variation in serum T_4 concentration for the group indicated

Effects of the GTG lesion are shown in figures 13a and 13b

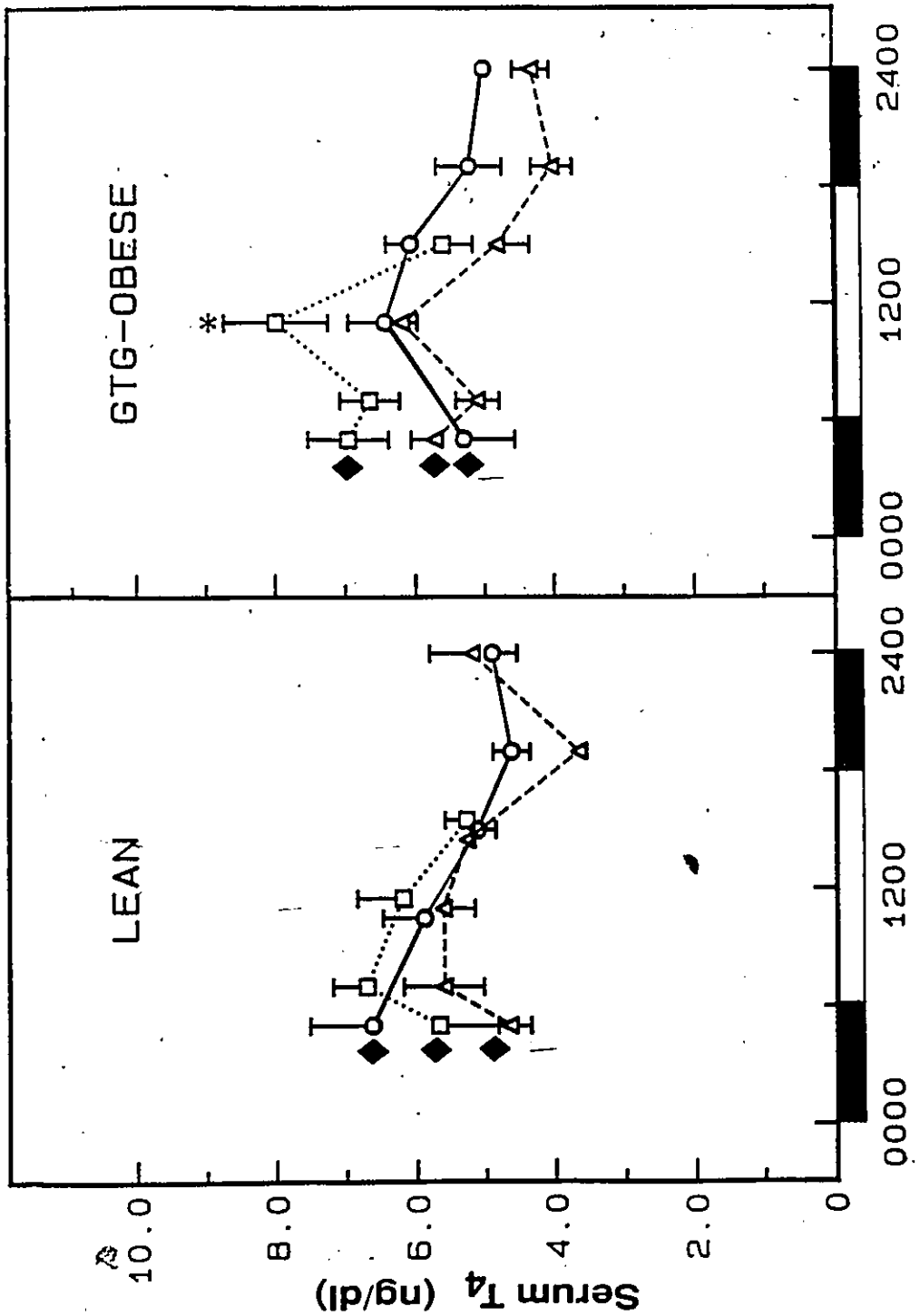

GTG-OBESE
LEAN
Serum T_4 (ng/dl)
10.0
8.0
6.0
4.0
2.0
0
0000
1200
2400
0000
1200
2400
TIME OF DAY

FIGURE 12

FIGURE 13a: SERUM T_4 CONCENTRATION OF LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW AND CAFETERIA-FED MICE

Data from figure 12 have been replotted to allow an easier assessment of the effect of GTG-lesion on serum T_4 concentration in mice eating the same diet. Chow- and cafeteria-fed mice were killed at the times shown. Food was presented to cafeteria-fed mice at 1500h. Lights were on between 0600h and 1800h. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for cafeteria-fed animals. Values represent means ± SE for 8 or more lean chow-, lean cafeteria and GTG chow-fed animals, and 5 or more GTG cafeteria-fed animals.

* Significant effect of diet, comparing the same type of mouse

◇ Significant effect of GTG-treatment, comparing mice eating the same diet

FIGURE 13b: SERUM T_4 CONCENTRATION OF LEAN AND GTG-OBESE MICE: EFFECT OF LESION ON CHOW-FED AND FOOD-RESTRICTED ANIMALS

Data have been replotted from figure 12. Rations were presented to food-restricted mice at 1500h. Note that the time points measured for these animals are different from those in the above figure. Unshaded bars represent results for mice fed chow diet. Shaded bars represent results for food-restricted mice. Values represent means ± SE for 8 or more mice. For further information see figure 7a.

FIGURE 13a

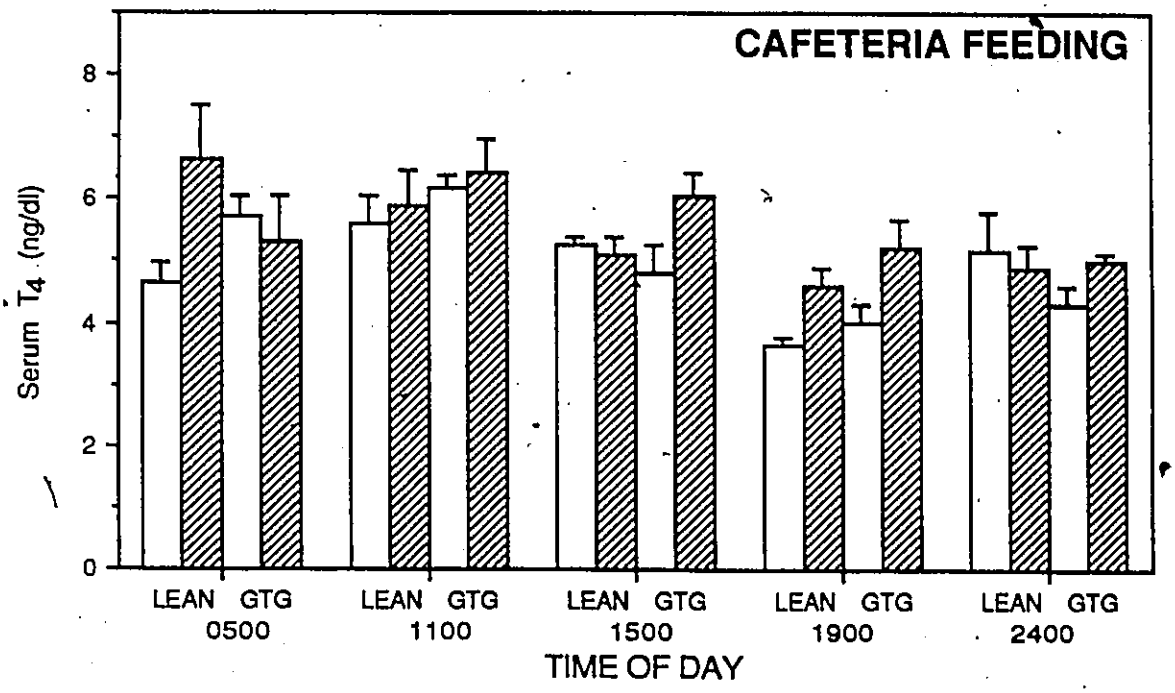
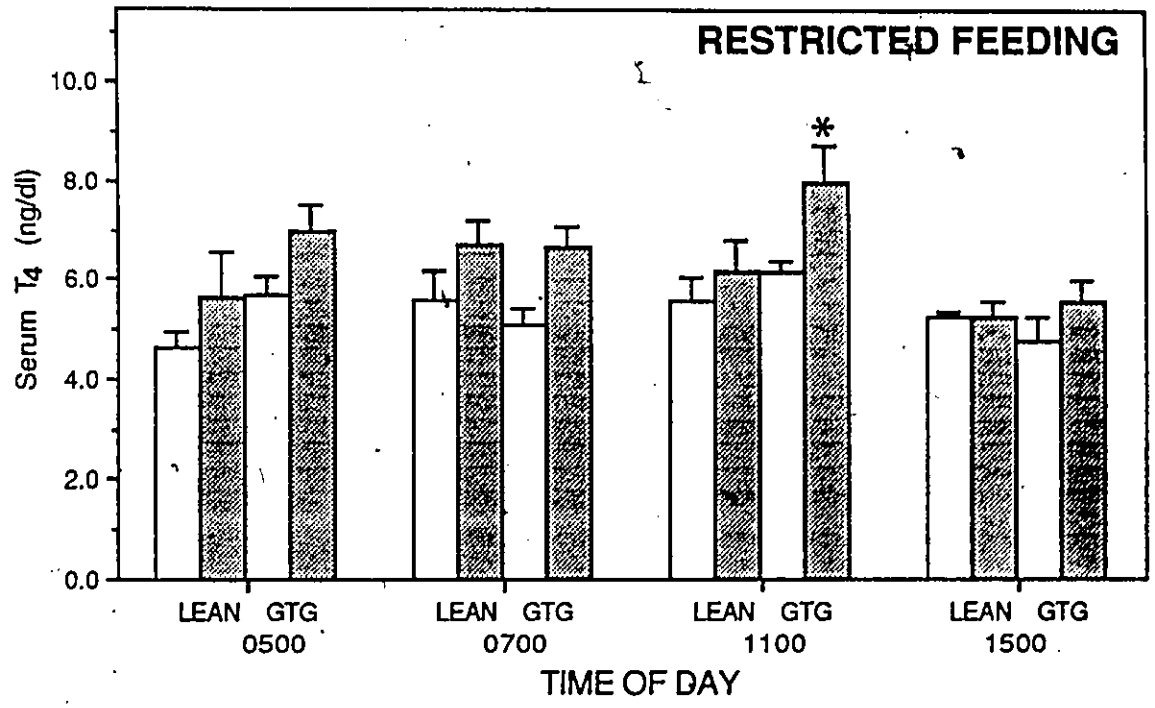


FIGURE 13b



PART II: BROWN ADIPOSE TISSUE OF GTG-OBESE MICE: EFFECT OF COLD

OBJECTIVES:

GTG-obese mice have been reported to survive exposure to 4° C (Davis and Mayer, 1954), and at 8 °C exhibit a large increase in mitochondrial GDP binding (Hogan and Himms-Hagen, 1983). This increase is, however, significantly lower than that of similarly-treated lean mice. Moreover, GTG-obese mice maintain a lower-than-normal body temperature for a large part of each day. Thus, a defect or alteration of thermoregulation may be present in these animals.

In two other animal models of obesity, the genetically obese (ob/ob) mouse (Kates and Himms-Hagen, 1986)and the Zucker (fa/fa) rat (Wu et al, 1987), an extreme cold sensitivity is associated with a marked reduction in the activity of BAT 5'-deiodinase.

The objectives of the second experiment were thus:

1. To compare the time course of activation of BAT thermogenesis in lean and GTG-obese mice, to determine whether a lesser activation at the single time measured previously was due to a reduced, or to a delayed increase in thermogenesis and thermogenic capacity.

2. To study the time course of cold-induced activation of BAT 5'-DII in the mouse, and to determine whether this activation is normal in the GTG-obese mouse.

EXPERIMENT 1: SURVIVAL OF GTG-OBESE MICE EXPOSED TO SEVERE COLD

METHOD:

Little experimental detail was to be found in the single previous report of survival of GTG-obese mice exposed to severe cold. Thus, a study was undertaken to further characterize this phenomenon. Three weeks after injection of GTG groups of lesioned animals and lean controls were placed in individual cages and were exposed to 4 °C. Rectal temperatures were recorded at hourly intervals for up to 7 hours, or until animals reached 20 °C or less. Hypothermic mice were returned to their home cages for recovery.

RESULTS

The tolerance of GTG-obese mice to severe cold was generally poor (figure 14a). Within 5 hours, five of eight exposed mice reached a core temperature of 20 °C or less, and would not have survived if left in the cold room. Only two GTG-obese mice endured seven hours of cold exposure and at the end of the study one of these was near hypothermia. The majority of lean mice (six out of eight) remained normothermic throughout the experiment (figure 14b). Surprisingly, two lean mice became hypothermic within six hours of acute exposure to 4 °C.

FIGURE 14a: RECTAL TEMPERATURES OF GTG-OBESE MICE ACUTELY EXPOSED TO 4 °C

Singly housed mice were exposed to 14 °C and rectal temperatures were measured hourly for 7 hours, or until temperatures reached 20 °C. Hypothermic mice were removed from the cold and allowed to recover. Each line is the record for one mouse.

FIGURE 14b: RECTAL TEMPERATURES OF LEAN MICE ACUTELY EXPOSED TO 4 °C

For details refer to legend for figure 14a.

FIGURE 14a

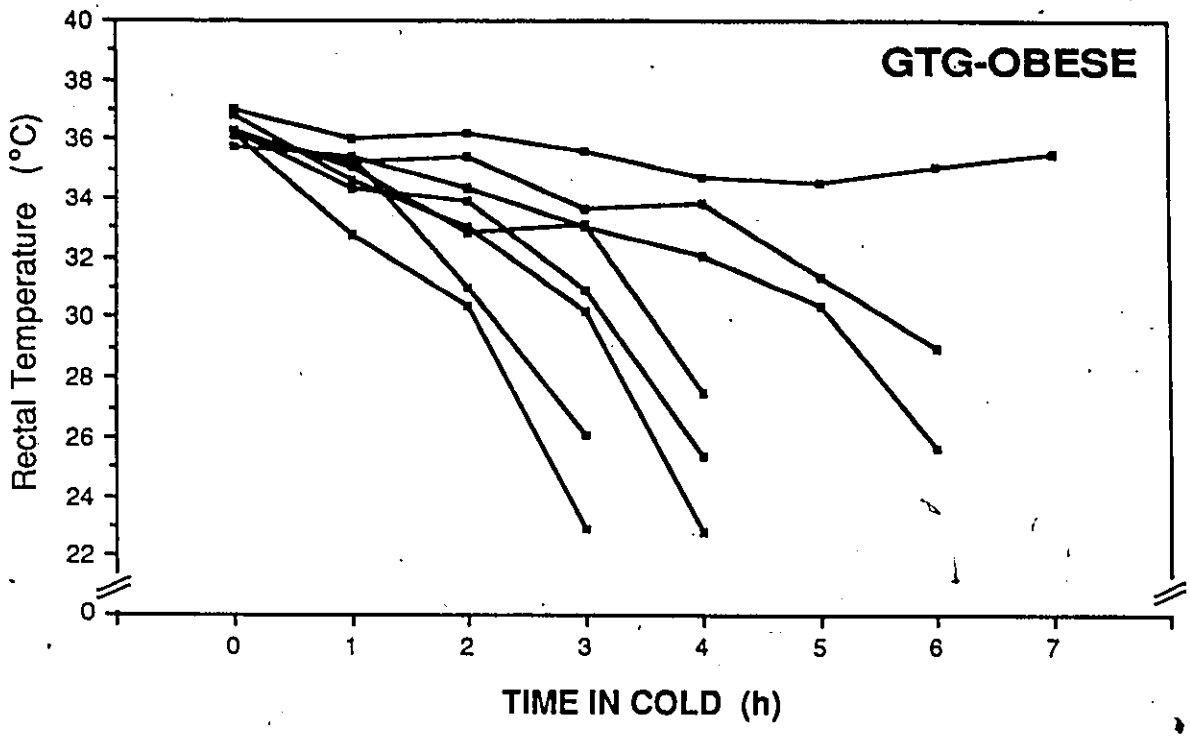
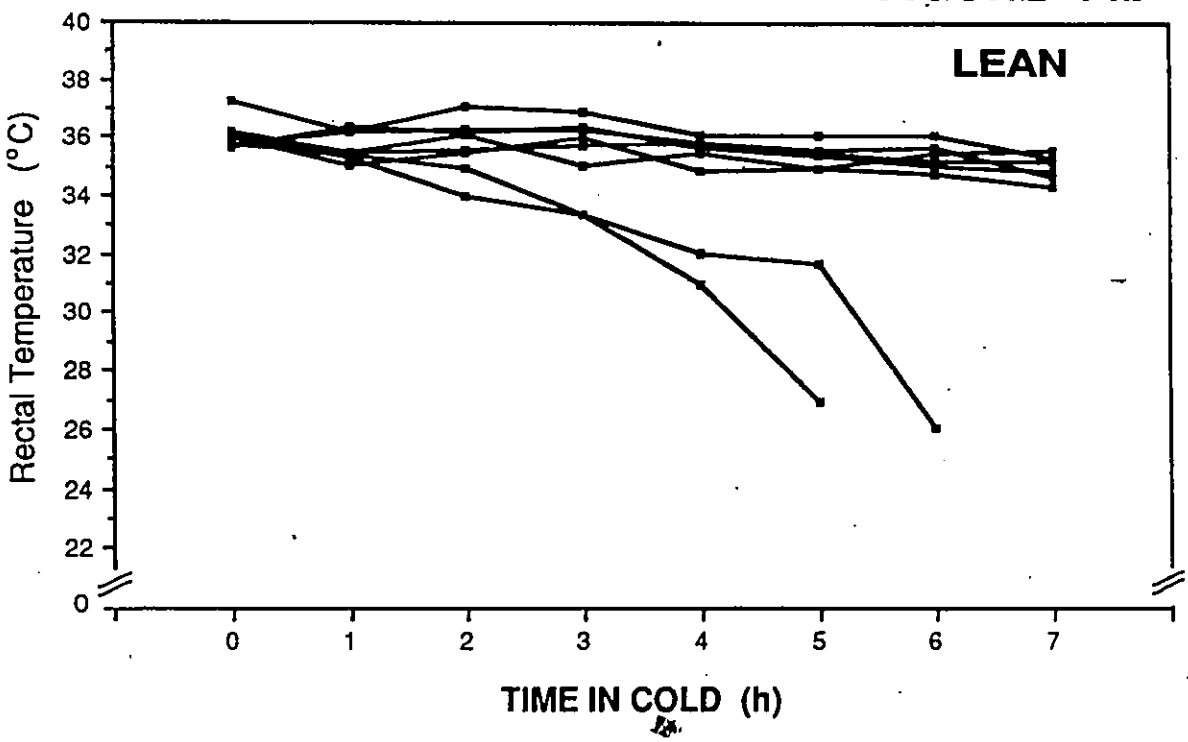


FIGURE 14b



EXPERIMENT 2: COLD EXPOSURE OF GTG-OBESE MICE: THERMOGENIC ACTIVATION AND 5'-DEIODINASE ACTIVITY

METHOD:

Due to the unexpected cold-sensitivity of GTG-obese mice, further investigations were carried out under conditions of milder cold (14 °C). Treatment of animals prior to cold exposure was identical to that described in part 1, except that mice received chow diet throughout the experiment. At 9-11 weeks of age a group of mice received GTG-induced lesions. Beginning two weeks after injection, animals were housed in separate cages and were exposed to 14 °C for 2, 7, 12, or 24 hours, or were acclimated to 14 °C for two weeks. A group of control animals, representing 0 hours of exposure to cold, were housed individually at 26.5 °C for 24 hours prior to sacrifice. In all cases, exposure was begun at 0700h.

After exposure, mice were sacrificed in the cold room, and BAT was quickly removed. Measurements of BAT protein, mitochondrial GDP binding, BAT 5'-DII activity, and serum T_3 and T_4 determinations were carried out as described for part one.

Results were analyzed using 2-way ANOVA, with obesity and time of exposure as the two treatments. Individual treatment effects were evaluated using Dunnett's test and simple means analysis (Kirk, 1968).

RESULTS:

1. THERMOGENIC ACTIVATION OF BAT

Cold exposure of lean and GTG-obese mice caused a linear increase in GDP-binding to isolated BAT mitochondria, suggesting a linear increase in thermogenic activation of the tissue in the first 24 hours of exposure (figure 15). The highest level of binding was achieved in both types of mice after the 2-week acclimation to cold. GDP-binding was significantly lower in GTG-obese mice at all time points. This difference appeared to result from a lower basal activation of BAT in these animals, since cold-induced increases above the warm-acclimated zero-point level were similar to those seen in lean mice.

2. BAT THYROXINE 5'-DEIODINASE ACTIVITY

Exposure to cold caused a large increase in specific and total activity of BAT 5'-DII in lean and GTG-obese groups (figure 16). Nonetheless, enzyme activation was both delayed and attenuated in GTG-obese animals. While lean mice reached maximum BAT 5'-DII activity after 7 hours of cold-exposure, GTG-obese mice required 12 hours to reach their highest level. Moreover, maximum 5'-DII activity was significantly lower in GTG-obese mice. In both groups, enzyme activity began to decline within twenty-four hours of cold exposure, and returned to basal levels after two weeks of cold acclimation.

FIGURE 15: BAT MITOCHONDRIAL GDP-BINDING OF LEAN AND GTG-OBESE MICE EXPOSED OR ACCLIMATED TO 14 °C

Singly-housed mice were exposed to cold for 2, 7, 12, or 24 hours, or were acclimated for two weeks. In each case, cold exposure was begun at 0700h. Values represent means ± SE for 8 mice.

SYMBOLS:

—○— **LEAN**

—●— **GTG-OBESE**

* Significant effect of exposure or acclimation to cold in mice of the same type

◇ Significant difference between lean and GTG-obese mice treated in the same way

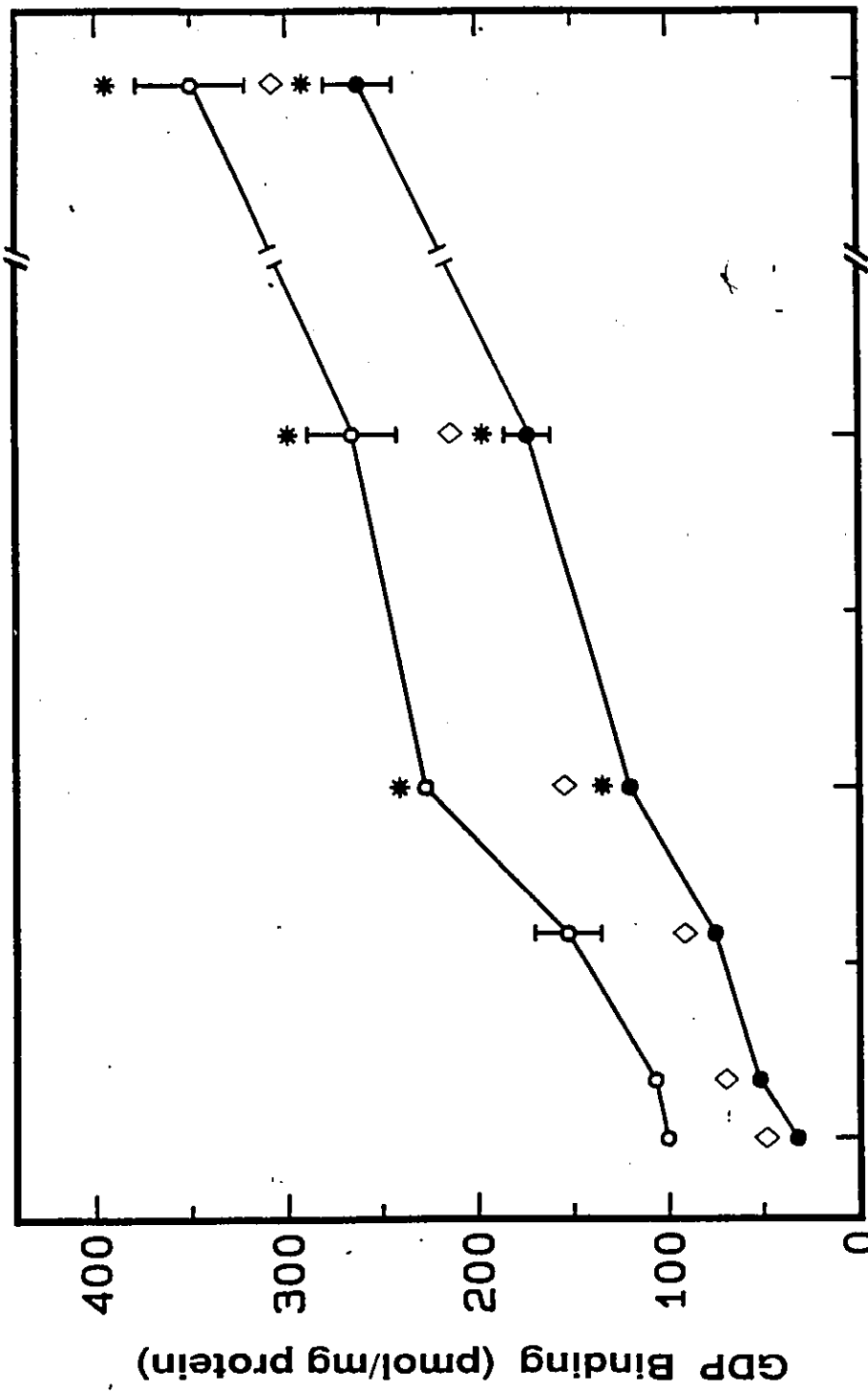

GDP Binding (pmol/mg protein)
400
300
200
100
0
0
12
24
14
Hours
Days
EXPOSURE TO 14 °C

FIGURE 15

FIGURE 16: **TOTAL AND SPECIFIC ACTIVITIES OF BAT T_4 5'-DEIODINASE IN LEAN AND GTG-OBESE MICE EXPOSED OR ACCLIMATED TO 14 °C**

Singly housed mice were exposed to cold for 2, 7, 12, or 24 hours, or were acclimated for two weeks. In each case, cold exposure was begun at 0700h. Values represent means ± SE for 8 mice

SYMBOLS:

—○— **LEAN**

—●— **GTG-OBESE**

* Significant effect of exposure or acclimation to cold in mice of the same type

◇ Significant difference between lean and GTG-obese mice treated in the same way

FIGURE 16

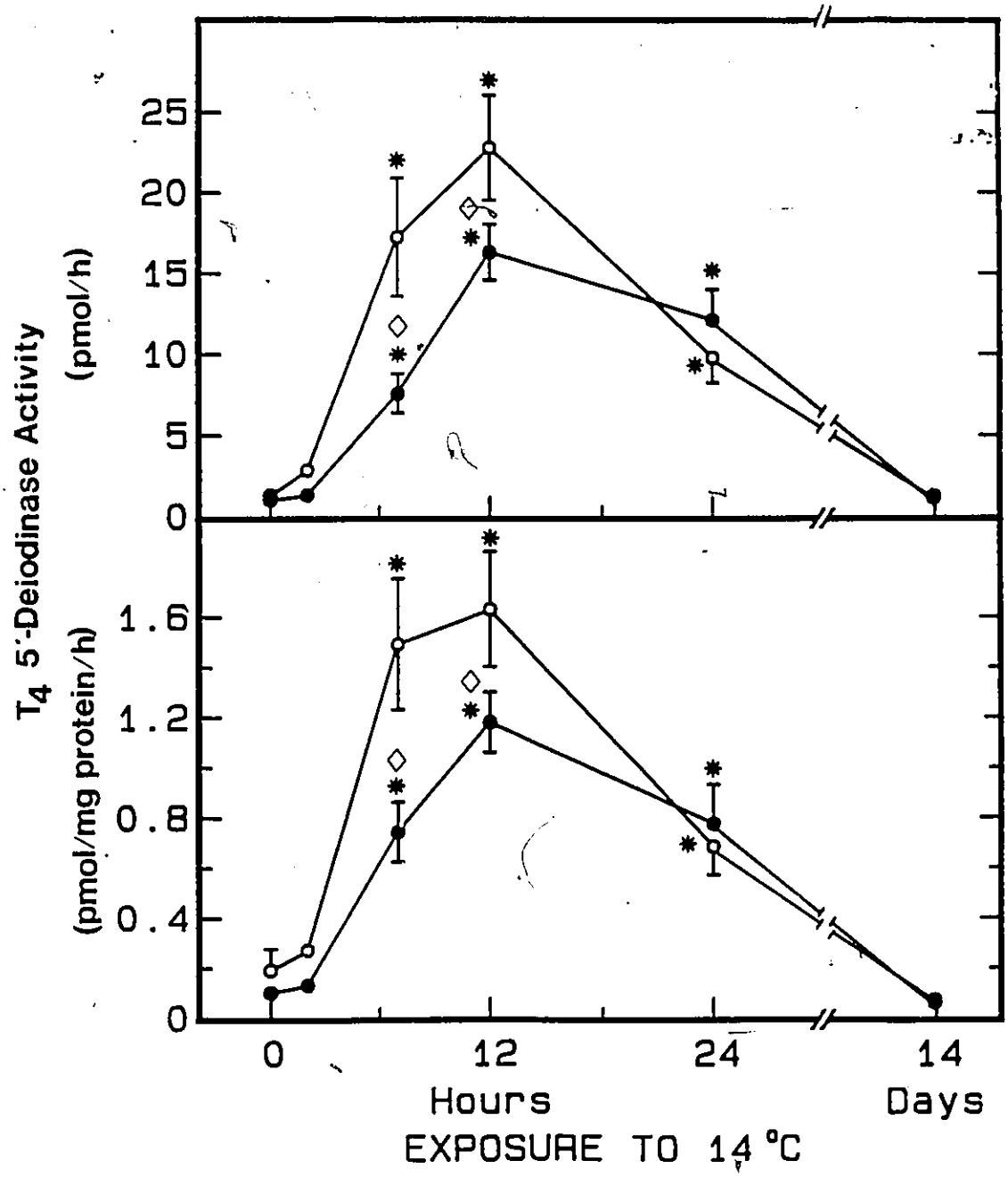

T_4 5'-Deiodinase Activity
(pmol/h)
25
20
15
10
5
0
(pmol/mg protein/h)
1.6
1.2
0.8
0.4
0
0
12
24
14
Hours
Days
EXPOSURE TO 14 °C

FIGURE 17: **SERUM THYROID HORMONE CONCENTRATIONS IN LEAN AND GTG-OBESE MICE EXPOSED OR ACCLIMATED TO 14 °C**

Singly housed mice were exposed to cold for 2, 7, 12, or 24 hours, or were acclimated for two weeks. In each case, cold exposure was begun at 0700h. Values represent means ± SE for 8 mice.

SYMBOLS:

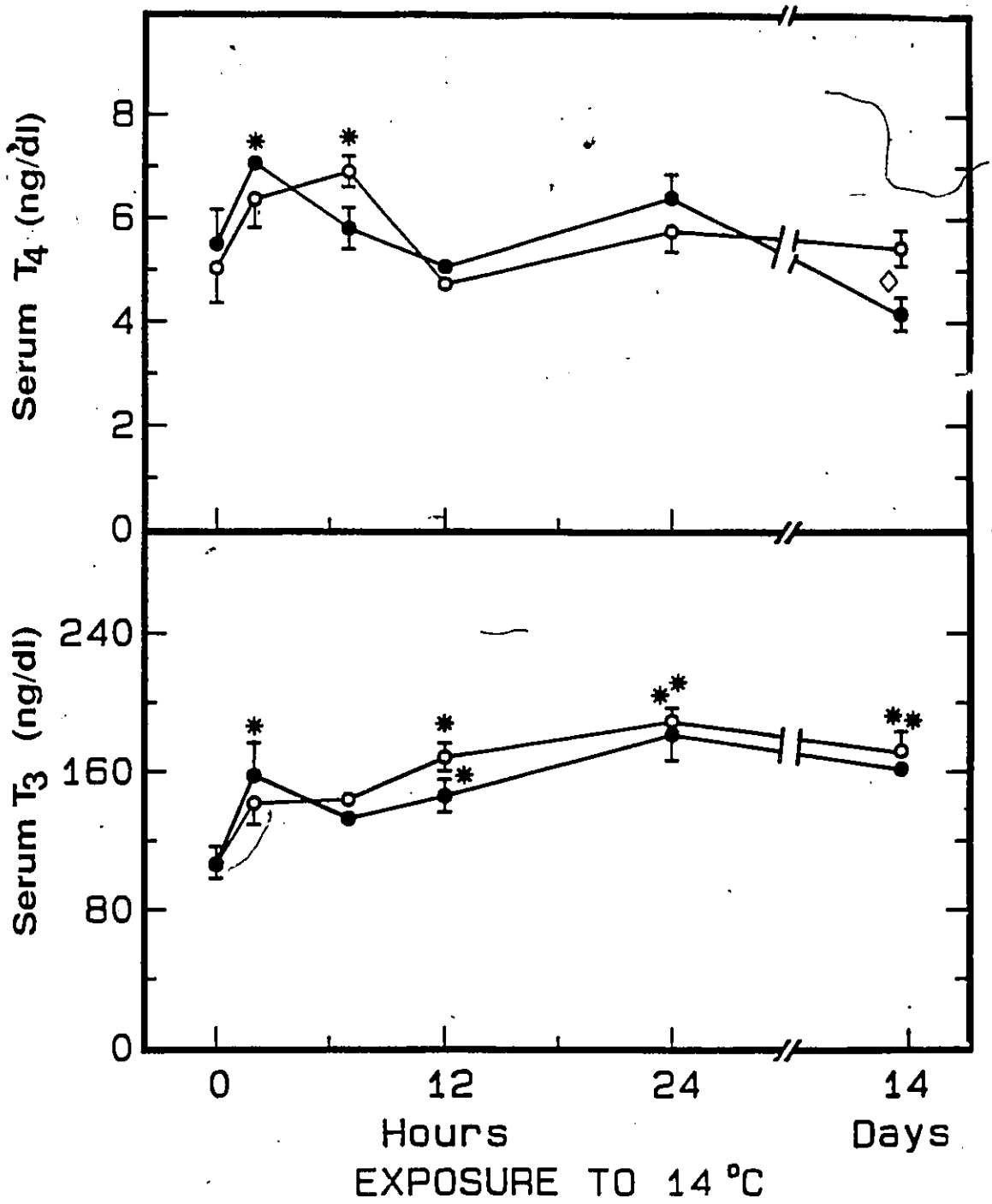
—○— **LEAN**

—●— **GTG-OBESE**

* Significant effect of exposure or acclimation to cold in mice of the same type

◇ Significant difference between lean and GTG-obese mice treated in the same way

FIGURE 17


Serum T_4 (ng/dl)
8
6
4
2
0
Serum T_3 (ng/dl)
240
160
80
0
0
12
24
14
Hours
Days
EXPOSURE TO 14 °C

3: THYROID HORMONES

Cold exposure resulted in an elevation of serum T_3 in both lean and GTG-obese mice (figure 17). This effect was detected within 12 hours of exposure and persisted through acclimation. A transient increase in serum T_3 occurred in GTG-obese mice at 2 hours of exposure; a corresponding increase in lean mice did not reach statistical significance.

Both lean and GTG-obese mice had an early, transient increase in the level of serum T_4 (figure 17). This effect occurred more rapidly in the GTG-obese group. After acclimation, serum T_4 was significantly reduced in GTG-obese mice, compared to lean cold-acclimated animals.

4: RECTAL TEMPERATURE

No change in rectal temperature was observed in lean mice exposed or acclimated to cold (table 3). Rectal temperature was significantly reduced in GTG-obese mice after 2 and 7 hours of cold exposure (compared to warm-exposed GTG-obese mice) and was lower than that of lean mice for the first 12 hours of exposure.

5: GROWTH OF BAT

Total BAT protein was significantly reduced in warm-acclimated GTG-obese mice with respect to their lean controls (table 4). Acclimation to cold promoted growth of

TABLE 3: BODY WEIGHTS AND RECTAL TEMPERATURES OF LEAN AND GTG-OBESE MICE EXPOSED OR ACCLIMATED TO 14 °C

Values represent means ± SE for 8 mice. In each case cold-exposure was begun at 0700h. Body weights were measured on the day prior to sacrifice. Rectal temperatures were measured immediately after mice were killed.

SYMBOLS:

* * Significant effect of exposure or acclimation to cold in mice of the same type

* ◇ Significant difference between lean and GTG-obese mice treated in the same way

	LEAN		GTG-OBESE	
	BODY WEIGHT (g)	RECTAL TEMPERATURE (°C)	BODY WEIGHT (g)	RECTAL TEMPERATURE (°c)
TIME IN COLD				
0h	19.7 ± 0.49	36.3 ± 0.24	25.4 ± 0.88 ◇	36.8 ± 0.15
2h	19.9 ± 0.45	35.9 ± 0.38	25.7 ± 0.79 ◇	35.0 ± 0.15 *◇
7h	20.6 ± 0.79	36.0 ± 0.24	27.0 ± 0.72 ◇	35.2 ± 0.23 *◇
12h	19.5 ± 0.40	37.2 ± 0.16	27.3 ± 0.84 ◇	35.9 ± 0.43 ◇
24h	20.8 ± 0.23	36.1 ± 0.38	27.7 ± 1.23 ◇	36.0 ± 0.34
2 wk	20.8 ± 0.60	36.9 ± 0.33	25.6 ± 0.66 ◇	37.2 ± 0.14

TABLE 3

TABLE 4: TISSUE WEIGHTS AND BAT PROTEIN IN LEAN AND GTG-OBESE MICE EXPOSED OR ACCLIMATED TO 14 °C

Values represent means ± SE for 8 mice.

SYMBOLS:

* * Significant effect of exposure or acclimation to cold in mice of the same type

* ◇ Significant difference between lean and GTG-obese mice treated in the same way

	LEAN			GTG-OBESE		
TIME IN COLD	WAT (mg)	BAT (mg)	BAT PROTEIN (mg)	WAT (mg)	BAT (mg)	BAT PROTEIN (mg)
0h	537 ± 71.4	249 ± 27.5	12.5 ± 0.57	972 ± 164.7 ◇	494 ± 44.5 ◇	9.6 ± 0.70 ◇
2h	443 ± 61.7	234 ± 13.5	10.3 ± 0.97	738 ± 58.0 ◇	501 ± 33.9 ◇	9.7 ± 0.39
7h	642 ± 69.8	259 ± 19.9	11.4 ± 1.22	958 ± 43.4 ◇	438 ± 20.8 ◇	10.4 ± 0.62
12h	483 ± 41.7	239 ± 13.0	13.5 ± 0.50	972 ± 70.1 ◇	388 ± 22.7 *◇	13.8 ± 0.49 *
24h	538 ± 103.5	256 ± 16.9	14.4 ± 0.40	1159 ± 142.6 ◇	395 ± 37.7 *◇	16.6 ± 0.94 *◇
2 wk	364 ± 75.6	374 ± 18.2 *	18.0 ± 1.01 *	587 ± 46.6 *	476 ± 23.6	19.7 ± 0.53 *

TABLE 4

BAT in both lean and GTG-obese mice (increase in BAT protein). Growth of BAT was apparent in the GTG-obese group within 12 hours of exposure; within twenty-four hours BAT protein was significantly greater for GTG-obese mice than for 24-hour-exposed lean mice. This difference was abolished by further cold acclimation.

The wet weight of BAT was greater in GTG-obese mice at all time points (table 4). This difference was presumably due to an accumulation of lipid in the tissue, since (except for the 24-hour point) BAT protein of GTG-obese mice was equal to or less than that of corresponding lean mice. BAT wet weight was decreased in GTG-obese mice by 12 and 24 hours of cold exposure, reflecting a decrease in fat content, but returned to previous levels upon acclimation. Cold acclimation of lean mice caused a decrease in the wet weight of BAT, indicating a reduction in fat content, as total protein levels were increased in this group.

At all time points GTG-obese mice were heavier than lean animals (table 3) and had a greater mass of gonadal WAT during cold exposure. Cold acclimation caused a reduction in WAT of GTG-obese animals, but was without effect in lean mice.

CHAPTER IV

DISCUSSION

The main objective of part I of this study was to assess the thermogenic state of BAT at different times of day in GTG-obese mice fed chow, restricted, or cafeteria diet. The specific goals were to determine whether DIT is activated at any time of day in GTG-obese mice fed a cafeteria diet, and to see whether an altered BAT thermogenic activity contributes to the high metabolic efficiency of the food-restricted GTG-obese mouse. Finally, I wished to characterize circadian changes in BAT 5'-deiodinase activity and to assess the effects of GTG lesioning upon this enzyme.

The principal finding of the first experiment is that cafeteria-feeding results in a daily, short-lived activation of thermogenesis in the BAT of GTG-obese mice, as suggested by the increased GDP binding observed in these animals at the afternoon and early evening time points. This transient activation could account for the growth of BAT seen previously in these animals (Hogan and Himms-Hagen, 1983) and in the present study. Brief daily exposure of mice to cold has been shown to promote growth of BAT at a level which equals or exceeds that produced by chronic cold exposure (Heldmaier, 1975). It is possible that brief daily diet-induced sympathetic activation promoted growth of BAT in cafeteria-fed GTG-obese mice in a similar manner.

Despite their capacity for DIT, thermogenic activity was

suppressed in GTG-obese mice for most of the day and night. Earlier reports of a lack of effect of cafeteria feeding on NATR (Zaror-Behrens and Himms Hagen, submitted) and GDP-binding to isolated BAT mitochondria (Hogan and Himms-Hagen, 1983) are in keeping with this finding, since these studies were carried out during the early light phase when DIT is absent in GTG-obese mice. The demonstration of thermogenic activation at one time of day suggests that a defective control, rather than destruction of the pathways for activation of DIT is responsible for the predominantly low thermogenic activity in the BAT of these animals.

It is not clear from the present experiment why DIT is activated normally in GTG-obese mice (as compared to lean cafeteria-fed mice) at the feeding time only. This observation can perhaps be related to the "palatability" or "tastiness" of the diet, since mice killed at the 1500h time point had both seen and tasted the food prior to sacrifice. Recent reports have suggested that feeding of a palatable meal, that is, one that is tasty and visually pleasing, increases the activity of the sympathetic nervous system, and may account for a significant proportion of meal-induced thermogenesis (LeBlanc et al, 1984, LeBlanc and Brondel, 1985, Diamond et al, 1985). A meal of identical composition presented in an unappealing manner (blended and desiccated into the form of a biscuit) fails to produce this activation. Palatability-induced thermogenesis is observed

upon presentation of the food, has a short duration (40 minutes), and is associated with an increase in plasma noradrenaline (LeBlanc et al, 1984). The initial increase in heat production is followed by a second, more prolonged component of meal-induced thermogenesis which is unrelated to the palatability of the meal. Thus it is possible that a short-lived, palatability-related component of DIT is intact in the GTG-obese mouse, producing the increase in GDP-binding at the mealtime. The subsequent, prolonged component may be defective, so that DIT is suppressed in these animals for the remainder of the day. Confirmation of this idea will require further study of the role of palatability in DIT in the mouse, and its relationship to brown adipose tissue.

Both anticipation of a meal and the oro- and viscerosensory stimuli associated with eating have been implicated as mediators of palatability-induced thermogenesis (Diamond et al, 1985). In the present experiment, all of these variables could be involved since the animals had learned to expect the meal, and had seen and tasted the food prior to sacrifice. However, anticipatory responses have been shown to depend upon an intact VMH-vagal-visceral pathway (Storlien, 1985). Therefore, for the GTG-obese mouse, factors associated with eating must presumably be the important ones. An exaggerated response to the orosensory properties of a palatable diet has been

reported in animals with medial hypothalamic lesions (Sclafani and Kirchgessner, 1986), and the results of the present experiment would certainly be consistent with such a response in the GTG-obese mouse.

Diet-induced activation of BAT was seen in lean cafeteria-fed mice throughout most of the day. It is likely that this activation resulted from a change in the composition of the diet, since total energy intake was not increased. The proportion of fat chosen by cafeteria-fed animals was 12-15 times higher than the fat content of the standard chow, and the proportion of protein was slightly less than half that of chow. In the rat, both high fat and low protein diets are known to stimulate DIT, as assessed by changes in NATR (Young et al, 1985, Schwartz et al, 1983), energetic efficiency (Rothwell et al, 1983a), metabolic rate (Rothwell and Stock, 1983b) and GDP-binding to isolated BAT mitochondria (Rothwell and Stock, 1987). Thus, although similar studies have not been performed for mice, it is possible that either the increased fat or the decreased protein concentration consumed by cafeteria-fed mice produced the changes observed in GDP binding in these animals. Stimulation of BAT thermogenesis may also have occurred as a consequence of an enhanced palatability of the cafeteria diet, compared with the standard chow (LeBlanc et al, 1985, Diamond et al, 1985).

It is interesting that cafeteria-feeding increased both

body weight and gonadal WAT in lean and GTG-obese mice without increasing total energy consumption. This is again presumably an effect of the high fat content of the diet, since the energy cost of fat deposition is much lower when lipid is derived directly from the diet, rather than from de novo fatty acid synthesis (Rothwell and Stock, 1983a). A slight weight gain without hyperphagia has been previously reported in cafeteria-fed mice (Kates and Himms-Hagen, 1986). The composition of the diet chosen by these animals was similar to that reported here. However, in another report, a substantial hyperphagia was observed in cafeteria-fed mice (Hogan, 1983). In this respect, the cafeteria-fed mouse may somewhat resemble the golden hamster, which becomes fatter when fed a high-fat diet, but which may or may not overeat (Triandafillou et al, 1984, Wade, 1982).

It is noteworthy that while GTG-obese mice were hyperphagic for the duration of the experiment, food intake was clearly regulated in these animals, since only small differences in caloric intake were observed from week to week. In addition, caloric intake was similar for chow and cafeteria-fed GTG-obese animals for all but the first intake period. Thus, the hyperphagia of the GTG-obese mouse appears to result not from a lack of regulation of food intake, but rather from regulation at an elevated level.

The circadian rhythm in BAT mitochondrial GDP binding in cafeteria-fed lean mice differs somewhat from that seen in

rats, in which binding is maximal in the late dark phase and mid-morning (Rothwell et al, 1983b). This discrepancy may result from differences in the time of food provision, since the time of presentation may affect the pattern of food intake, and thus conceivably could influence the rhythm in BAT thermogenic activation. As well, a species difference in binding rhythm is apparent since no circadian variation has been observed for chow-fed rats (Rothwell et al, 1983b) while a marked circadian rhythm is now shown for chow-fed mice.

It is intriguing that in both chow-fed mice and cafeteria fed rats, GDP-binding is lowest through most of the dark phase, when the greatest feeding activity occurs (Petersen, 1978, Becker and Kissileff, 1974). It is possible that a high level of physical activity in feeding animals during the early dark phase results in an increase in body temperature (Zaror-Behrens and Himms-Hagen, submitted, Himms-Hagen, 1985b, and present experiment) that is sufficient to suppress BAT metabolism. Exercise has been reported to suppress BAT thermogenesis in both rats (Arnold et al, 1986) and mice (Richard and Trayhurn, 1984). It is not clear, however, why this phenomenon is not observed in cafeteria-fed mice. Since DIT is promoted by hyperphagia, from the point of view of energy balance one would expect the tendency to suppress DIT to be inversely related to the degree of hyperphagia, yet GDP-binding remained high

throughout the dark phase in cafeteria-fed mice, while caloric intake was not increased. In contrast, GDP-binding is suppressed in the dark phase in cafeteria-fed rats, though energy consumption may rise by 70-80% (Rothwell and Stock, 1983a). This species difference may involve differences in activity or eating patterns, diet composition or palatability effects, and clearly cannot be answered without further study. It must be remembered, as outlined in the introduction, that the GDP binding technique permits an assessment of BAT thermogenic state, and is a function of both the concentration of binding sites, and the extent to which these are exposed and accessible to binding (Himms-Hagen, 1983). Thus, the increased binding observed in cafeteria-fed mice may have resulted from a noradrenaline-stimulated unmasking of binding sites on the UCP, from an increase in the concentration of this protein, or from both. In rats, at least, cafeteria-diet-induced increases in UCP concentration have been demonstrated (Falcou et al, 1985, Ashwell et al, 1984, Nedergaard et al, 1984).

BAT mitochondrial GDP-binding was reduced by food-restriction of lean mice; this reduction may reflect both a masking of sites and a decrease in the concentration of UCP (Trayhurn and Jennings, 1986), or a masking of sites only (Desautels, 1985). No circadian rhythm was observed in GDP-binding of food-restricted mice, despite the large changes in body temperature which occurred in these animals during

the period of study. This result is in agreement with a report of suppressed sympathetic nervous system activity in food-restricted mice during the arousal period (Zaror-Behrens and Himms-Hagen, submitted). Both results are surprising, however, since BAT thermogenic activation has been demonstrated in mice during arousal from torpor (Himms-Hagen, 1985b). The discrepancy between these studies probably arises from differences in the stimulus for arousal. Where activation of BAT has been observed (Himms-Hagen, 1985b) a very rapid arousal (12-30 minutes) was stimulated by moving torpid mice out of their home cages. Where evidence of BAT activation was not observed (Zaror-Behrens and Himms-Hagen, submitted, and present experiment) the stimulus for arousal was the expectation of food, and rewarming occurred over several hours. It is possible therefore, that under the conditions of the present experiment rewarming occurred through a transient activation of BAT thermogenesis that was not measured at the chosen time points. Alternatively, rewarming may have been accomplished through a small but prolonged increase in BAT thermogenesis.

In contrast to lean mice, food-restriction did not result in further suppression of the low BAT mitochondrial GDP-binding of GTG-obese mice. It should be noted that further suppression would be both possible and measurable, based on the very low values recorded for obese chow-fed

animals in the early dark phase. This finding is in agreement with previous reports of suppressed sympathetic avtivity in BAT of food-restricted lean, but not food-restricted GTG-obese mice (Zaror-Behrens and Himms-Hagen, submitted) and of lack of diet-sensitivity of the sympathetic nervous-system of GTG-obese mice (Young and Landsberg, 1980).

The cause of the reduced GDP-binding in BAT of chow-fed GTG-obese mice is not known. It may be that this reduction occurs through an action of corticosterone, since administration of this hormone is known to suppress BAT thermogenic activation (Galpin et al, 1983), probably through a centrally-mediated reduction in sympathetic activity to the tissue (Vander Tuig et al, 1984, York et al, 1985). While corticosterone levels are essentially normal in the GTG-lesioned mouse in the dynamic phase of its obesity (Saito and Bray, 1983), as discussed in the introduction, it is possible that an abnormally high central sensitivity to glucocorticoid is present in this animal. Such an enhanced sensitivity has been reported for two models of genetic obesity, the fatty Zucker rat (fa/fa) and the ob/ob mouse (Freedman et al, 1986, Holt et al, 1983, Tokuyama and Himms-Hagen, 1987).

The circadian variation in body temperature and effects of diet on it were the same in this study as previously recorded (Zaror-Behrens and Himms-Hagen, submitted), with

cafeteria-feeding increasing and food-restriction decreasing body temperature for different proportions of the day. By far the greatest temperature variation was seen in food restricted mice. While these groups maintained their temperatures above 31 °C, the value which has been defined as the threshold of torpor, (Hudson and Scott, 1979), it must be remembered that this value was chosen arbitrarily for convenience of measurement. Whether or not defined as torpor, the temperature depression of up to 6 °C observed in food-restricted mice for a substantial proportion of each day would necessarily have contributed to the energetic efficiency of these animals, and would therefore have helped to minimize their weight loss.

Several factors may be involved in the temperature differences observed in food-restricted and cafeteria-fed groups, compared to their respective controls. Serum T_3 is elevated in cafeteria-fed mice (Rothwell and Stock, 1979, Hogan, 1983 and present experiment) and decreased in food-restricted mice (present experiment) and thus may be responsible for alterations of obligatory thermogenesis (Himms-Hagen, 1983). As well, a role for BAT in the production of these differences is generally supported by the changes observed in mitochondrial GDP-binding of cafeteria-fed mice. The reduced temperatures of food-restricted lean mice can be attributed to a lesser thermogenic activation of BAT, and to a reduction in serum

T_3. Similarly, chow-fed GTG-obese mice displayed a lower GDP-binding at each time of day measured. Serum T_3 was reduced in food-restricted GTG-obese mice, which may explain their lower body temperatures compared to obese controls. However, none of the variables measured in the present experiment account for the large reduction of body temperature in food-restricted GTG-obese mice compared to their lean counterparts, since food intake, serum T_3, and BAT mitochondrial GDP-binding did not differ in the two groups. Thus a third factor must be invoked to explain the much lower body temperatures of the food-restricted GTG-obese mouse. One possibility is that GTG-obese mice maintain a lower activity level than their lean counterparts. A reduction in activity-induced thermogenesis has been reported for another type of obese animal, the genetically obese (ob/ob) mouse (Dauncey, 1986). Alternatively, lower body temperatures may have been achieved in these animals through changes in thermoregulatory mechanisms (for example, decreased thermal conductance due to decreased peripheral blood flow) in response to an altered central temperature set point (Ganong, 1985).

In confirmation of previous reports (Kates et al, 1986, Wu et al, 1987), no effect of cafeteria feeding was seen on BAT 5'DII activity of lean mice at any time of day. It would seem, therefore, that BAT 5'DII activity and

thermogenic state are under separate control, since GDP-binding was increased in these animals. This finding brings to view an interesting question. The activity of BAT 5'DII is known to be increased by noradrenaline, whether through exogenous administration, or through cold-induced sympathetic activation (Silva and Larsen, 1983, Jones et al, 1986). Cafeteria feeding also results in sympathetic activation and release of noradrenaline from nerve terminals supplying BAT (Landsberg and Young, 1983, Zaror-Behrens and Himms-Hagen, submitted). How noradrenaline can stimulate BAT 5'DII activity in the one case, yet be without effect in the other is not known. It is unlikely that the different effects result from changes in adrenergic receptor content, since α_1- adrenergic receptors, through which NA acts to stimulate 5'DII (Silva and Larsen, 1983), are increased in both cafeteria-fed and cold-exposed rats. Althouqh the α:β receptor ratio is increased in BAT of cold-exposed (Rothwell et al, 1986, Raasmaja et al, 1984a), but not cafeteria-fed animals (Rothwell et al, 1986, Raasmaja et al, 1984b), it is unlikely that the effects of NA differ in the two situations due to an altered adrenergic receptor ratio, since 5'DII activity is acutely stimulated by a single injection of noradrenaline (Jones et al, 1986). For the same reason, it is doubtful that a factor present in cold-exposed animals is required in addition to noradrenaline for stimulation of enzyme activity. A more likely explanation for this

phenomenon is that a factor, possibly hormonal, operates in cafeteria-fed animals to prevent a NA-induced increase in BAT 5'DII activity. Inhibition of BAT 5'DII activity by growth hormone has been described (Silva and Larsen, 1986a), however the effects of cafeteria feeding on growth hormone levels are not known. Alternatively, the lack of stimulation of 5'DII activity by NA in cafeteria-fed animals could result from the decrease in plasma insulin in these animals (Rothwell and Stock, 1983a). Insulin has been shown to stimulate BAT 5'DII activity (Silva and Larsen, 1986b, Mills et al, 1987), and insulin levels are reduced in cafeteria-fed rats (Rothwell et al, 1983b). At present, however, it can only be concluded that the regulation of BAT 5'DII is complex, involving both neural and hormonal components, and presents many avenues for further study.

The activity of BAT 5'DII was for the most part unaltered by food restriction of lean mice despite the suppression of NATR (Zaror-Behrens and Himms-Hagen, 1986) and low mitochondrial GDP-binding (present experiment) produced by this treatment. This observation again suggests that a separate control of BAT 5'DII activity and thermogenic state operates under these conditions. Similarly, no difference was observed in 5'DII activity between chow-fed lean and GTG-obese mice, despite marked differences in BAT mitochondrial GDP-binding. It is surprising that 5'DII activity was elevated by cafeteria-

feeding in GTG-obese mice in the late dark phase only. The reason for this observation is unclear.

The circadian variation observed in BAT 5'DII activity has not been previously reported. This rhythm did not parallel the daily variation in mitochondrial GDP-binding, again suggesting a control that is independent of sympathetic activation. Several hormones have been implicated in the regulation of BAT 5'DII (Silva and Larsen, 1986a, 1986b). Among these, insulin has been shown to produce an increase in BAT 5'DII activity in a manner that is unrelated to sympathetic nervous system activity. A circadian variation in plasma insulin has been reported for rats (Rothwell et al, 1983b) and is similar to the variation in BAT 5'DII activity in these animals (Park and Himms-Hagen, unpublished). Thus it is conceivable that the rhythm in BAT 5'-deiodinase activity could be driven by the rhythm in plasma insulin.

It is noteworthy that the rhythm of BAT 5'DII activity is also similar to the circadian variation in serum corticosterone concentration (Saito and Bray, 1983), and like the deiodinase activity is unaltered in GTG-obese mice in the dynamic phase of obesity. The effects of corticosterone upon BAT 5'DII have not been well characterized, although an elevated BAT 5'DII activity in hypophysectomized animals is reportedly not suppressed by corticosterone treatment (Silva and Larsen, 1986a). The

role of corticosterone in the regulation of BAT 5'-deiodinase activity deserves further study.

The objectives of part II of this work were to compare the time course of activation of BAT thermogenesis and of BAT 5'DII activation in lean and GTG-obese mice. In the first experiment, the ability of these animals to survive exposure to severe cold was studied. The hypothermia displayed by GTG-obese mice in this experiment was surprising, since thermoregulation in severe cold is reportedly normal in these animals (Davis and Mayer, 1954). The discrepancy in these results may be due to differences in the dosage of GTG used to induce obesity, or in the length of recovery period allowed between GTG administration and cold exposure. These details were not reported in the earlier study. From the results of the second experiment, in which the time course of activation of BAT thermogenesis and 5'-DII activation were measured, it is clear that the GTG-obese mouse is capable of activating BAT thermogenesis when exposed to milder cold, since the increase in BAT mitochondrial GDP-binding paralleled that seen in lean mice. As well, the cold-induced increase in BAT protein occurred even earlier in GTG-obese mice, indicating a normal adaptive growth of the tissue in these animals.

In the cold-acclimated state, the level of GDP-binding was several-fold that of warm-acclimated mice, presumably reflecting both unmasking of sites and an increase in the

concentration of uncoupling protein (Desautels et al, 1986). Despite a normal increase, the absolute level of GDP-binding was always somewhat lower in the GTG-obese mice; this difference was particularly marked in warm acclimated animals, where binding was only 30% of normal. The lower GDP-binding of warm-acclimated GTG-obese mice, and the smaller response to cold at one time of exposure have been observed previously (experiment one, and Hogan and Himms-Hagen, 1983). The reason for this difference is not clear. At 26 °C, measurements of NATR in chow-fed GTG-obese mice indicate a normal sympathetic activity (Zaror-Behrens and Himms-Hagen, submitted). NATR has not been measured in cold-exposed GTG-obese mice, but the time course of increase in GDP-binding in these animals, parallel to that of lean mice, suggests a normal sympathetic activation in the cold. Thus, in both warm and cold-exposed GTG-obese mice, the presence of, or perhaps lack of some factor other than noradrenaline is presumably responsible for the lower GDP-binding. This factor appears to affect the "basal" thermogenic state of BAT in these animals, rather than stimulated thermogenic activation, since differences in GDP-binding between lean and GTG-obese mice were about the same at each point studied.

It is possible that the lower baseline of thermogenic activation accounts for the cold-sensitivity observed in GTG-obese mice in the second experiment; in severe cold, the

initial low level of BAT activation may have been insufficient to allow survival for long enough to complete the activation process. Similarly, a low baseline of thermogenic activity may account for the hypothermia observed in two lean mice.

The time course of increase in BAT 5'DII activity in cold-exposed mice has not been previously reported. After an initial peak at 12 hours of cold exposure, the activity of this enzyme declined, returning to normal levels with acclimation. This finding is surprising, since mitochondrial GDP-binding and tissue growth, also under the control of noradrenaline, continued to increase after 5'DII levels had returned to minimal values. Thus, a dissociation of 5'DII activity and thermogenic state is apparent in cold-acclimated animals. However, this dissociation is unlike that observed in cafeteria-fed mice in which mitochondrial GDP-binding (experiment one), as well as sympathetic activity (Zaror-Behrens and Himms-Hagen, submitted) are increased without an apparent stimulation of 5'DII activity. In mice, the time course of cold-induced sympathetic activation is similar to that of deiodinase activity, with a large initial increase, followed by a decline to normal values in 2-week cold-acclimated animals (Zaror-Behrens and Himms-Hagen, 1983). Thus, the question here is not why deiodinase activity is low in cold-acclimated mice, but rather why GDP-binding and tissue protein levels are so high

in the absence of sympathetic activation.

In the rat, the time course of increase in mitochondrial GDP-binding in cold-acclimating animals has been correlated with a concurrent rise in the concentration of mitochondrial UCP (Trayhurn et al, 1987). A similar phenomenon appears to occur in the mouse, based on the large increase in UCP content that is observed after cold acclimation (Desautels, Dulos, and Mozaffari, 1986). Recent reports have suggested that the acute cold-induced increase in UCP is dependent upon intracellular conversion of T_4 to T_3 by BAT 5'DII (Bianco and Silva, 1987, Bianco and Silva, in press). In these experiments, thyroidectomized rats showed a five-fold smaller increase in levels of UCP than euthyroid rats; this response could be normalized using small doses of T_4. In contrast, UCP levels were raised to only 50% of euthyroid values by T_3 and this response required supraphysiological doses. The large increase in UCP levels produced by cold-exposure has been shown to require a nuclear T_3 receptor saturation of greater than 80% (Bianco and Silva, in press). Since the nuclear T_3 receptor saturation of warm-acclimated euthyroid rats is estimated to be near 70% (Bianco and Silva, 1987b) it has been suggested that activation of BAT 5'DII is necessary to produce the high nuclear T_3 concentration required for a cold-induced increase in UCP. However, if the time course of increase in UCP concentration is similar in mice to that observed in rats (a significant

increase only after 48 hours of cold exposure, Trayhurn et al, 1987), the results of the present experiment suggest that some, if not all of this increase must occur during the period of declining 5'DII activity. Thus, while a deiodinase-dependent increase in nuclear T_3 receptor saturation is required for a normal rise in UCP levels of acutely cold-exposed rats, (Bianco and Silva, in press), the present results suggest that in cold-acclimating mice a continued synthesis may be promoted through a T_3-independent mechanism. Alternatively, after nuclear T_3 levels are raised by the initial increase in 5'DII concentration, the subsequent lower 5'DII activity may be sufficient to maintain nuclear T_3 concentration at the level required for receptor saturation. Similarly, after a large initial sympathetic stimulation, a tonic level of noradrenaline release may be adequate for continued growth of BAT. Although NATR in cold-acclimated mice is similar to that in mice acclimated to 26°C, a tonic sympathetic activation is apparent in these animals, since NATR is reduced in mice acclimated to 33°C, the thermoneutral zone of this species (Zaror-Behrens and Himms-Hagen, 1983).

The time course of increase of BAT 5'DII activity in cold-exposed mice differs from that of both rats and hamsters (Kopecky et al, 1986). In rats, a similar pattern is seen for the first 24 hours of cold exposure, but after acclimation, enzyme activity is still markedly elevated. In

hamsters, the increase in 5'DII activity occurs much more slowly; maximal specific activities are observed no sooner than three days after cold exposure, and total activity continues to increase after 4 weeks of cold-acclimation. These differences suggest distinct functions for BAT 5'DII in the three species. In rats and hamsters, changes in BAT 5'DII activity correlate with changes in serum T_3 (Kopecky et al, 1986, Park and Himms-Hagen, submitted); thus, in these animals, BAT 5'DII may be important not only for local generation of T_3, but also for provision of this hormone to the general circulation. Indeed, an increase in T_3 content of venous blood from interscapular BAT has been observed in cold-stressed rats (Fernandez, Mampel, Villaroya and Iglesias, 1987). In mice, the initial elevation in T_3 level occurs prior to the increase in 5'DII actitity; moreover serum T_3 remains elevated irrespective of declining 5'DII levels in cold-acclimating animals. In mice, therefore, 5'DII may have a more specific function in local generation of T_3 for stimulation of UCP synthesis, with the cold-induced increase in serum T_3 resulting either from enhanced thyroidal secretion, or from BAT-independent peripheral deiodination. It would be interesting to determine whether the prolonged increase in 5'DII activity in cold-acclimating rats and hamsters has the sole purpose of production of T_3 for the general circulation, or whether this activity is also necessary to maintain thermogenic

activation of BAT in these animals.

The initial increase in BAT 5'DII activity was lower than normal in GTG-obese mice exposed to cold. Thus, a slight attenuation of responsiveness was observed at this time. This is not to say, however, that a normal maximal level of 5'DII activity could not be reached in GTG-obese mice, since the increase may have occurred more slowly in these animals, reaching a peak at a time point not studied. By 24 hours the elevation in activity of this enzyme was the same in obese and lean mice. Thus, GTG-obese mice demonstrated a several-fold increase in 5'DII activity within 12 hours of cold-exposure, and in this way differ from both the genetically obese (ob/ob) mouse (Kates and Himms-Hagen, 1985) and the Zucker obese (fa/fa) rat (Wu et al, 1987), both of which display a severe impairment of cold-induced BAT 5'DII elevation.

It is doubtful that the attenuation of 5'DII activity seen in the cold-exposed GTG-obese mouse results from a difference in sympathetic activation, considering the parallel increase in mitochondrial GDP-binding of control and GTG-obese animals. Therefore one must again invoke a hormonal factor to explain an altered regulation of this enzyme, although the nature of this factor in cold-exposed animals remains to be determined. As well, the consequences of this attenuation are not clear, since the changes in rectal temperature of cold-exposed GTG-obese mice were

small. It may be that the delay in cold-activation of BAT 5'DII assumes a more important role when GTG-obese mice are submitted to severe cold, as in the second experiment.

Several features of the abnormal energy balance of the GTG-obese mouse are similar to those of the mouse made obese by neonatal treatment with monosodium glutamate (MSG). Although, unlike the GTG-lesioned mouse, the MSG-obese mouse is not hyperphagic, both animals thermoregulate at a reduced body temperature during the dark and early light phase, both succumb to hypothermia when exposed to severe cold, and both can adapt when exposed to a milder cold stimulus (Tokuyama and Himms-Hagen, 1986, and present results). In addition, both animals have lesions in the hypothalamic arcuate nucleus (Olney, 1969, 1971, Takahashi, 1987, Tanaka et al, 1978, Powley and Prechtl, 1986). While arcuate lesions are the major consequence of MSG treatment, they are a minor but reproducible consequence of GTG administration. Thus, though the obesity-inducing effects of the two treatments appear to be fairly distinct (Smith,1983) it is possible that their common effect upon the arcuate nucleus is responsible for the altered thermoregulation and sensitivity to severe cold seen in both types of mice.

CHAPTER V

CONCLUSION

Contrary to the results of previous, more limited studies, diet-induced activation of BAT thermogenesis is not abolished in the GTG-obese mouse. Instead, DIT occurs only transiently each day, and is suppressed for the remainder of each 24-hour period. This finding suggests that control of DIT is not destroyed by the GTG lesion, but rather is altered, perhaps in relation to the altered thermoregulation displayed by this animal.

It is likely that the increased metabolic efficiency of the food-restricted GTG-obese mouse results from its ability to thermoregulate at a subnormal body temperature. This ability appears to rely upon a factor or factors apart from suppression of BAT thermogenesis. Furthermore, the reduced basal thermogenic activity of the chow-fed GTG-obese mouse may account for its sensitivity to severe cold; cold-induced thermogenic activation occurs normally in this animal. In addition, and in contrast to two genetically obese species, the regulation of BAT 5'DII activity is largely normal in the GTG-obese mouse.

The obesity of the GTG-lesioned mouse is the consequence both of an increased food intake, and of a reduced energy

expenditure. While the contribution of an altered thermogenic activity to the elevated weight gain of this animal has not been quantified in the present study, it is nonetheless clear that an altered regulation of DIT, and suppression of basal activation of BAT contribute to the development of its obesity. Thus, the elevated energetic efficiency demonstrated by the GTG-obese mouse appears to result partly, but not entirely, from an altered regulation of BAT thermogenic activity.

APPENDIX A

LIST OF ABBREVIATIONS

ADP	adenosine 5'-diphosphate
AN	arcuate nucleus
ATP	adenosine 5'-triphosphate
Bq	becquerel
Ci	curie
BAT	brown adipose tissue
CHO	carbohydrate
CIT	cold-induced thermogenesis
DIT	diet-induced thermogenesis
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
5'DII	T_4 5'-deiodinase
GDP	guanosine 5'-diphosphate
GTG	goldthioglucose
HEPES	N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid
LH	lateral hypothalamus
MH	medial hypothalamus
MSG	monosodium glutamate
NA	noradrenaline
NATR	noradrenaline turnover
NST	nonshivering thermogenesis
PTU	propylthiouracil

ABBREVIATIONS, CONTINUED

PVN	paraventricular nucleus
SE	standard error
SN	supraoptic nucleus
T_b	body temperature
T_3	triiodothyronine
T_4	thyroxine
UCP	uncoupling protein
VMH	ventromedial hypothalamus
VMN	ventromedial nucleus
WAT	white adipose tissue

APPENDIX B1

MENUS FOR CAFETERIA DIETS

MENU	
1	Chocolate Wafer Walnut Cheddar Cheese Oatmeal Cookie
2	Chocolate Wafer Almond Processed Cheese Shortbread Cookie
3	Chocolate Wafer Pecan Swiss Cheese Chocolate Chip Cookie

In addition, cafeteria-fed animals received chow ad libitum

APPENDIX B2

COMPOSITION OF CAFETERIA FOODS

	ENERGY (Kcal/g)	PROTEIN %	CHO %	FAT %
CHOCOLATE WAFER	5.5	7.9	52.1	32.1
WALNUT	6.54	15.0	15.6	64.4
ALMOND	5.47	18.6	19.6	54.1
PECAN	6.96	9.4	13.0	73.0
CHEDDAR CHEESE	3.98	25.0	2.1	32.2
PROCESSED CHEESE	3.32	20.0	7.6	25.0
SWISS CHEESE	3.21	21.8	4.6	24.3
OATMEAL COOKIE	4.44	6.1	76.2	17.7
SHORTBREAD COOKIE	5.00	7.1	65.7	22.9
CHOCOLATE CHIP COOKIE	4.73	5.5	70.0	20.9
CHOW	4.34	23.5	52.3	4.5

Compositions of the cafeteria foods were obtained from Bowes and Church's Food Values of Portions commonly used (Pennington and Church, 1980). Values for chow were obtained from the Ralston Purina Company.

% refer to percent of nutrient per gram wet weight of food.

APPENDIX C

CURRICULUM VITAE

NAME: JUDY M. ELEY

DATE OF BIRTH: 4th March, 1960

PLACE OF BIRTH: Vancouver, British Columbia

CITIZENSHIP: Canadian

EDUCATION:

University of Ottawa
Ottawa, Ontario
M.D., registered 1986 - present

University of Ottawa
Ottawa, Ontario
M.Sc. (Biochemistry) registered 1984-present

University of British Columbia
Vancouver, British Columbia
B.Sc. (Agr) Animal Nutrition, 1982

Pleasant Valley Senior Secondary School
Armstrong, British Columbia (grades 11-12)

AWARDS:

H.M. King Prize in Animal Science, 1982
Wilfred Sadler Memorial Gold Medal, 1982
NSERC Postgraduate Scholarship, 1984-1986
University of Ottawa Supplemental Research Scholarship, 1984-1986

EXPERIENCE:

Student research assistant, Department of Animal Science, UBC, 1982-1983

Lab demonstrator - Biochemistry 2936
University of Ottawa, 1984

Lab demonstrator - Biochemistry 3946
University of Ottawa, 1985

PUBLICATIONS AND ABSTRACTS:

(1) Full Papers:

Eley, J.M., and Himms-Hagen, J., Brown adipose tissue of mice with goldthioglucose-induced obesity: Abnormal circadian control. Manuscript in preparation.

Eley, J.M., and Himms-Hagen, J., Attenuated response to cold in brown adipose tissue of goldthioglucose-obese mice. Manuscript in preparation.

(2) Abstracts:

Eley, J.M., and Himms-Hagen, J., Delayed cold-induced activation of thyroxine 5'-deiodinase in brown adipose tissue (BAT) of goldthioglucose-obese mice (Abstract of poster presented at XXX Congress of International Union of Physiological Sciences, Vancouver, British Columbia, July 1986)

Himms-Hagen, J., and Eley, J., Circadian rhythms in brown adipose tissue of lean and goldthioglucose obese mice: effect of diet. Fed. Proc. 46(4):1337 (Abstract 5953)

REFERENCES

Anand, B.K., and Brobeck, J.R., (1951) Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med. 24:123-140.

Anand, B.K., Chhina, G.S.J., Sharma, K.N., Dua, S., and Singh, B. (1964) Activity of single neurons in the hypothalamic feeding centers: effects of glucose. Am. J. Physiol. 207(5):1146-1154.

Anand, B.K., and Dua, S., (1955) Feeding responses induced by electrical stimulation of the hypothalamus in cats. Indian J. Med. Res. 43:113-122.

Arnold, J., Leblanc, J., Cote, J., Lalonde, J., Richard, D. (1986) Exercise suppression of thermogenesis in 24- and 4°C-acclimated rats. Can. J. Physiol Pharmacol 64:922-926.

Ashwell, M., Jennings, G., Richard, D., Stirling, D.M., and Trayhurn, P., (1983) Effect of acclimation temperature on the concentration of the mitochondrial 'uncoupling' protein measured by radioimmunoassay in mouse brown adipose tissue. FEBS Letters, 161(1):108-112.

Ashwell, J., Rothwell, N.J., Stirling, D., Stock, M.J., and Winter, P.D., (1984) Changes in mitochondrial uncoupling protein and GDP-binding in brown adipose tissue of cafeteria-fed rats. Proc. Nutr. Soc. 43:148A.

Bailey, P., and Bremer, F., (1921) Experimental diabetes insipidus and genital atrophy. Endocrinology 5, 761-762.

Barnard, T., Mory, G., Nechad, M., (1980) Biogenic amines and the trophic response of brown adipose tissue. In: Biogenic Amines in Development, eds. H. Parvez, S. Parvez, pp 391-439. Amsterdam: Elsevier.

Becker, E.E., and Kissileff, H.R., (1974) Inhibitory controls of feeding by the ventromedial hypothalamus. Am. J. Physiol 226(2):383-396.

Beckstead, R.M., and Norgren, R., (1979) An autoradiographic examination of the central distribution of the trigeminal, facial, glossopharyngeal, and vagal nerves in the monkey. J. Comp. Neurol. 184:455-472.

Bianco, A.C., and Silva, J.E., (1987a) Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue.

J. Clin. Invest. 79:295-300.

Bianco, A.C., and Silva, J.E., (1987b) Nuclear 3,5,3'-triiodothyronine (T_3) in brown adipose tissue: receptor occupancy and sources of T_3 as determined by in vivo techniques. Endocrinology 120:55-62.

Bianco, A.C., and Silva, J.E., Optimal response of key enzymes and uncoupling protein to cold in brown adipose tissue depends on local triiodothyronine generation. Am. J. Physiol. in press.

Bray, G.A., and York, D.A., (1979) Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol. Rev. 59:719-809.

Brecher, G., Laqueur, G.L., Cronkite, E.P., Edelman, P.M., and Schwartz, I.L. (1965) The brain lesion of goldthioglucose obesity. J. Exp. Med. 121:395-402.

Brecher, G., and Waxler, S.H., (1949) Obesity in albino mice due to a single injection of goldthioglucose. Proc. Soc. Exp. Biol. and Med. 70:498-501.

Brooks, S.L., Rothwell, N.J., and Stock, M.J., (1982) Effects of diet and acute noradrenaline treatment on brown adipose tissue development and mitochondrial purine-nucleotide binding. Q. J. Exp. Physiol. 67:259-268.

Brown, D.F., and Viles, J.M., (1982) Systemic phlorizin prevents gold thioglucose necrosis in the ventromedial hypothalamus. Brain Res. Bull. 8(4):347-351.

Bryant, K.R., Rothwell, N.J., Stock, M.J., and Stribling, D., (1983) Idenditication of two mitochondrial GDP-binding sites in rat brown adipose tissue. Biosci. Rep. 3:589-598.

Bukowiecki, L.J., (1984) Mechanisms of stimulus-calorigenesis coupling in brown adipose tissue. Can. J. Biochem. Cell Biol. 62:623-630.

Bukowiecki, L., and Collet, A.J., (1983) Regulation of brown adipose tissue metabolism. J. Obesity Weight Regulation, 2:29-53.

Caffyn, Z.E.Y., (1971) Ultrastructural changes induced in the hypothalamus of the mouse by goldthioglucose. Br. J. Exp. Path. 52:517-523.

Caffyn, Z.E.Y., (1972a) Early vascular changes in the brain

of the mouse after injections of goldthioglucose and bipiperidyl mustard. J. Path. 106:49-56.

Caffyn, Z.E.Y., (1972b) The inhibition of goldthioglucose and bipiperidyl mustard-induced hypothalamic lesions by anti-inflammatory agents. J. Path. 106:57-63.

Cannon, B., Nedergaard, J., and Sundin, U., (1981) Thermogenesis, brown fat and thermogenin. In: Survival in the Cold: Hibernation and Other Adaptations. Eds. X.J. Musacchia and L. Jansky, Elsevier North Holland, Inc., Amsterdam, pp 99-120.

Cooney, G.J., Newsholme, E.A., (1984) Does brown adipose tissue have a metabolic role in the rat? Trends in Biochemical Sciences 9:303-305.

Coscina, D.V., Chambers, J.W., Park, I., Hogan, S., Himms-Hagen, J., (1985) Impaired responses of brown adipose tissue in rats made obese with parasaggital hypothalamic knife cuts. Brain. Res. Bull. 14:585-593.

Dauncey, J. J., (1986) Activity-induced thermogenesis in lean and genetically obese (ob/ob) mice. Experientia 42:547-549.

Davis, T.R.A., and Mayer, J., (1954) Imperfect homeothermia in the hereditary-obese hyperglycemic syndrome of mice. Am. J. Physiol. 177:222-226.

Debons, A.F., Das, K.C., Fuhr, B., and Siclari, E., (1983) Anorexia after adrenalectomy in goldthioglucose-treated obese mice. Endocrinol. 112:1847-1851.

Debons, A.F., Das, K.C., Fuhr, B., and Siclari, E., (1982b) Inhibition by hypophysectomy of the hyperphagia and obesity following gold-thioglucose. Physiol. and Behav. 29:695-699.

Debons, A.F., Siclari, E., Das, K.C., and Fuhr, B., (1982) Gold-thioglucose-induced hypothalamic damage, hyperphagia, and obesity: dependence on the adrenal gland. Endocrinol. 110:2024-2029.

Debons, A.F., Silver, L., Cronkite, E.P., Horton, E.R., Johnson, H.A., Brecher, G., Tenzer, D., and Schwartz, I.L., (1962) Localization of gold in mouse brain in relation to gold-thioglucose obesity. Am. J. Physiol. 202(4):743-750.

Debons, A.F., Krimsky, I., From, A., and Cloutier, R.J., (1969) Rapid effects of insulin on the hypothalamic satiety center. Am. J. Physiol, 217:114-118.

Debons, A.F., Krimsky, I., From, A., and Cloutier, R.J., (1970a) Gold thioglucose induction of obesity: significance of focal gold deposits in hypothalamus. Am. J. Physiol. 219(5):1403-1408.

Debons, A.F., Krimsky, I., From, A., and Cloutier, R.J., (1970b) Site of action of goldthioglucose in the hypothalamic satiety center. Am. J. Physiol. 219(5):1397-1402.

Debons, A.F., Krimsky, I., From, A., Siclari, E., Maayan, M.L., Fani, K., and Jiminez, F.A., (1979a) Action of gold-thioglucose on pericapillary structures in the ventromedial hypothalamus. J. Pathol. 129:73-81.

Debons, A.F., Krimsky, I., From, A., and Siclari, E., (1979b) Mediation by serotonin of goldthioglucose-induced necrosis of the ventromedial hypothalamus. J. Pathol. 129:83-90.

Debons, A.F., Krimsky, I., Likuski, H.J., From, A., and Cloutier, R.J., (1968) Goldthioglucose damage to the satiety center: inhibition in diabetes. Am. J. Physiol. 214:652-658.

Debons, A.F., Zurek, L.D., Tse, C.S., and Abrahamsen, S., (1986) Central nervous system control of hyperphagia in hypothalamic obesity: dependence on adrenal glucocorticoids. Endocrinology 118:1678-1681.

De Laey, P., Dent, C., Terry, A.C., and Quinn, E.H., (1975) Food and water intake in gold thioglucose-induced obese Charles River mice. Arch. Int. Pharmacodyn. 213:145-162.

Depocas, F., and Behrens, W.A., (1977) Effects of handling, decapitation, anesthesia and surgery on plasma noradrenaline levels in the white rat. Can. J. Phys. Pharmacol. 55(2):212-219.

Desautels, M., (1985) Mitochondrial thermogenin content is unchanged during atrophy of BAT of fasting mice. Am. J. Physiol. 249:E99-E106.

Desautels, M., Dulos, R.A., and Mozaffari, B., (1986) Selective loss of uncoupling protein from mitochondria of surgically denervated brown adipose tissue of cold-acclimated mice. Biochem. Cell. Biol. 64: 1125-1134.

Desautels, M., and Himms-Hagen, J., (1979) Roles of noradrenaline and protein synthesis in the cold-induced

increase in purine nucleotide binding by rat brown adipose tissue mitochondria. Can. J. Biochem. 57:968-976.

Desautels, M., Zaror-Behrens, G., and Himms-Hagen, J., (1978) Increased purine nucleotide binding, altered polypeptide composition and thermogenesis in brown adipose tissue mitochondria of cold-acclimated rats. Can. J. Biochem. 56:378-383.

Diamond, P., Brondel, L., and LeBlanc, J., (1985) Palatability and post-prandial thermogenesis in dogs. Am. J. Physiol. 248:E75-E79.

Djazayery, A., Miller, D.S., and Stock, M.J., (1979) Energy balances in obese mice. Nutr. Metab. 23:357-367.

Drachman, R.H., and Tepperman, J., (1954) Aurothioglucose obesity in the mouse. Yale J. Biol. Med 26:394-409.

Edelman, P.M., Schwartz, L., Cronkite, E.P., Brecher, G., and Livingstone, L., (1965) The effect of hyperglycemia on hypothalamic gold uptake. J. Exp. Med. 121:403-413.

Falcou, R., Bouillaud, F., Mory, G., Apfelbaum, M., and Ricquier, D., (1985) Increase of uncoupling protein and its mRNA in brown adipose tissue of rats fed on 'cafeteria diet'. Biochem. J. 231:241-244.

Fernandez, J.A., Mampel, T., Villarroya, F., and Iglesias, R., (1987) Direct assessment of brown adipose tissue as a site of systemic tri-iodothyronine production in the rat. Biochem. J., 243:281-284.

Foster, D.O., (1984) Quantitative contribution of brown adipose tissue thermogenesis to overall metabolism. Can. J. Biochem. cell Biol. 62:618-622.

Foster, D.O., and Frydman, M.L., (1978) Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. Can. J. physiol. Pharmacol. 56: 110-122.

Freedman, M.R., Horwitz, B.A., and Stern, J.S., (1986) Effect of adrenalectomy and glucocorticoid replacement on development of obesity. Am. J. Physiol. 250:R595-R607.

Freedman, M.R., Stern, J.S., Reaven, G.M., and Mondon, C.E., (1986) Effect of adrenalectomy on in vivo glucose metabolism in insulin resistant Zucker obese rats. Horm. Metabol. Res. 18:296-298.

Galpin, K.S., Henderson, R.G., James, W.P.T., and Trayhurn, P., (1983) GDP binding to brown-adipose-tissue mitochondria of mice treated chronically with corticosterone. Biochem. J., 214:265-268.

Ganong, W.F., In: Review of Medical Physiology, (1985) eds. J. Lange and D. Lange, Lange Medical Publications, Los Altos, California, pp 201-203.

Girardier, L., (1983) Brown fat: an energy dissipating tissue. In: Mammalian Thermogenesis, Eds. L. Girardier and M.J. Stock, pp 50-98.

Gold, R.M., Jones, A.P., Sawchenko, P.E., and Kapatos, G., (1977) Paraventricular nucleus: critical focus of a longitudinal neurocircuitry mediating food intake. Physiol. Behav. 18:1111-1119.

Gray, G.F., and Liebelt, R.A., (1961). Food intake studies in gold-thioglucose-obese CBA mice. Texas Rep. Biol. Med. 19:80-88.

Graff, H., and Stellar, E., (1962) Hyperphagia, obesity, and finickiness. J. Comp. Physiol. Psychol. 55:418-424.

Hales, C.N., and Kennedy, G.C., (1964) Plasma glucose, non-esterified fatty acid and insulin concentrations in hypothalamic hyperphagic rats. Biochem. J. 90:620-624.

Heldmaier, G., (1975) The effect of short daily cold exposures on development of BAT in mice. J. Comp. Physiol. 98:161-168.

Hetherington, A.W., and Ranson, S.W., (1939) Experimental hypothalamico-hypophyseal obesity in the rat. Proc. Soc. Exp. Biol. Med. 41:465-466.

Hetherington, A.W., and Ranson, S.W., (1940) Hypothalamic lesions and adiposity in rats. Anat. Rec. 78:149-172.

Himms-Hagen, J., (1979) Brown adipose tissue thermogenesis, energy balance, and obesity. Can. J. Biochem. Cell Biol. 62:610-617.

Himms-Hagen, J., (1983a) Thyroid hormones and thermogenesis. In: Mammalian Thermogenesis, Eds.. L. Girardier and M.J. Stock, pp 141-177.

Himms-Hagen, J., (1983b) Brown adipose tissue thermogenesis in obese animals. Nutr. Rev. 41:261-267.

Himms-Hagen, J., (1984a) Brown adipose tissue thermogenesis, energy balance and obesity, Can. J. Biochem. Cell Biol. 62:610-617.

Himms-Hagen, J., (1984b) Brown adipose tissue thermogenesis as an energy buffer. Implications for obesity. New Engl. J. Med. 311(24):1549-1558.

Himms-Hagen, J., (1985a) Brown adipose tissue metabolism and thermogenesis. Ann. Rev. Nutr. 5:69-94.

Himms-Hagen, J., (1985b) Improved brown adipose tissue thermogenic function in food-restricted ob/ob mice: relation to occurrence of torpor. Am. J. Physiol. 248:E531-E539.

Himms-Hagen, J., Triandafillou, J., and Gwilliam, C., (1981) Brown adipose tissue of cafeteria-fed rats. Am. J. Physiol. 241:E116-E120.

Hogan, S., (1983) Brown adipose tissue in relation to energy balance. Ph.D. Thesis, University of Ottawa

Hogan, S., Coscina, D.V., and Himms-Hagen, J., (1982) Brown adipose tissue of rats with obesity-inducing ventromedial hypothalamic lesions. Am. J. Physiol. 243:E338-E344.

Hogan, S., and Himms-Hagen, J., (1983) Brown adipose tissue of mice with gold thioglucose-induced obesity: effect of cold and diet. Am. J. Physiol. 244:E581-E588.

Hogan, S., Himms-Hagen, J., and Coscina, D.V., (1985) Lack of diet-induced thermogenesis in brown adipose tissue of obese medial hypothalamic-lesioned rats. Physiol. Behav. 35:287-294.

Holiday, J.F., Fish, K.W., Churchill, B., and Marrazzi, M.A., (1982) Effects of bipiperidyl mustard (BPM) lesions on insulin hypoglycemic convulsions. Physio. Behav. 29(3):543-551.

Holt, S., and York, D.A., (1984) Effect of adrenalectomy on brown adipose tissue of obese (ob/ob) mice. Horm. Metab. Res. 16:378-379.

Holt, S., York, D.A., and Fitzsimons, J.R.R., (1983) The effects of corticosterone, cold exposure and overfeeding with sucrose on brown adipose tissue of obese Zucker rats (fa/fa). Biochem. J. 214:215-223.

Hudson, J. W., and Scott, I.M., (1979). Daily torpor in the

laboratory mouse Mus musculus, var. albino. Physiological Zoology 52:205-218.

Jacobsson, A., Nedergaard, J., and Cannon, B., (1986) α- and β-adrenergic control of thermogenin mRNA expression in brown adipose tissue. Bioscience Reports, 6(7):621-631.

Jones, R., Henschen, L., Mohell, N., and Nedergaard, J., (1986) Requirement of gene transcription and protein synthesis for cold and norepinephrine-induced stimulation of thyroxine deiodinase in rat brown adipose tissue. Biochimica et Biophysica Acta 889:366-373.

Joosten, H.F.P., and van der Kroon, P.H.W., (1974) Role of the thyroid in the development of the obese-hyperglycemic syndrome in mice (ob/ob). Metabolism, 23(5):425-436.

Kaplan, M.M., (1980) Thyroxine 5'-monodeiodination in rat anterior pituitary homogenates. Endocrinology 106:567.

Kaplan, M.M., and Yaskoski, K.A., (1980) Phenolic and tyrosyl ring deiodination of iodothyronines in rat brain homogenates. J. Clin. Invest. 66:551-562.

Kataoka, K., Danbara, H., Sunayashiki, K., Okuno, S., Sorimachi, M., Tanaka, M., and Chikamori, K., (1978) Regional and subcellular distribution of gold in brain of gold thioglucose-obese mouse. Brain Res. Bull. 3(3):257-263.

Kates, A-L., and Himms-Hagen, (1985) Defective cold-induced stimulation of thyroxine 5'deiodinase in brown adipose tissue of the genetically obese (ob/ob) mouse. Biochem. Biophys. Res. Commun. 130(1):188-193.

Kates, A-L., and Himms-Hagen, (1986) Thyroxine 5'-deiodinase in brown adipose tissue (BAT) of genetically obese (ob/ob) mice: effect of cold and cafeteria diet. (Abstract P-10) Fourth European Bioenergetics conference, Prague, Czechoslovakia.

Kates, A-L., Park, I.R.A., Himms-Hagen, J., and Kopecky, J., (1986) Abstract: Thyroxine 5'-deiodinase activity in brown adipose tissue of rats and mice: lack of effect of diet. Fed. Proc. 45:610 No. 2650.

Kirk, R.E., (1968) Experimental Design: Procedures For The Behavioral Sciences. Brooks/Cole Publishing Company, Belmont California.. pp 69-222.

Kopecky, J., Sigurdson, L., Park, I.R.A., and Himms-Hagen, J., (1986) Thyroxine 5'-deiodinase in hamster and rat brown adipose tissue: effect of cold and diet. Am. J. Physiol. 251:E1-E7.

La Noue, K.F., Strzelecki, R., Strzelecka, D., and Koch, C., (1986) Regulation of the uncoupling protein in brown adipose tissue. J. Biol. Chem. 261(1):298-305.

Landsberg, L., and Young, J.B., (1983) Autonomic regulation of thermogenesis. In: Mammalian Thermogenesis, Eds. L. Girardier and M.J. Stock, pp 99-140.

Leblanc, J., and Brondel, L., (1985) Role of palatability on meal-induced thermogenesis in human subjects. Am. J. Physiol. 248:E333-E336.

Leblanc, J., Cabanac, M., and Samson, P., (1984) Reduced postprandial heat production with gavage as compared with meal feeding in humans. Am. J. Physiol. 246:E95-E101.

Le Magnen, J., (1983) Body energy balance and food intake: a neuroendocrine regulatory mechanism. Physiological Reviews 63(1):314-386

Le Marchand, Y., Freychet, P., and Jeanrenaud, B., (1978) Longitudinal study on the establishment of insulin resistance in hypothalamic-obese mice. Endocrinol. 102:74-85.

Leonard, J.L., Mellen, S.A., and Larsen, P.R., (1983) Thyroxine 5'-deiodinase activity in brown adipose tissue. Endocrinology 112:1153-1155.

Leonard, J.L., and Rosenberg, I.N., (1980) Iodothyronine 5'-deiodinase from rat kidney: Substrate specificity and the 5'-deiodination of reverse triiodothyronine. Endocrinology 107:1376.

Leslie, R.A., Gwyn, D.G., and Hopkins, D.A., (1982) The central distribution of the cervical vagus nerve and gastric afferent and efferent projections in the rat. Brain Res. Bull. 8:37-43.

Liebelt, R.A., Sekiba, K., Ichinoe, S., and Liebelt, A.G., (1966) Persistent vaginal cornification in goldthioglucose-treated BALB/c/ki mice. Endocrinol. 78:845-854.

Liebelt, R.A., Sekiba, K., Liebelt, A.G., Perry, J.H., (1960) Genetic susceptibility to gold-thioglucose-induced obesity in mice. Proc. Soc. Exp. Biol. Med. 104:689-693.

Likuski, H.J., Debons, A.F., and Cloutier, R.R., (1967) Inhibition of goldthioglucose-induced hypothalamic obesity by glucose analogues. Am. J. Physiol. 212:669-676.

Lloyd, L.E., McDonald, B.E., and Crampton, E.W., (1978) In: Fundamentals of Nutrition, Ed. G.W. Salisbury, pp 396-423.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J., (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265-275.

Luby, J.L., Holliday, J.F., Fish, K.F., and Marazzi, M., (1981) Change in sensitivity to insulin hypoglycemic convulsions after gold thioglucose treatment: time course of development. J. Pharmacol. Exp. Ther. 219:258-267.

Marrazzi, M.A., McRae, W., and Brown, J.A., (1985) Thioglucose interactions with a "gold thioglucose-lesioned glucostat." J. Pharmacol. Exp. Ther. 234(3):804-813.

Marshall, N.B., Barrnett, R.J., and Mayer, J., (1955) Hypothalamic lesions in goldthioglucose-injected mice. Proc. Soc. Expl. Biol. and Med. 70:240-244.

Mayer, J., (1960) The hypothalamic control of gastric hunger contractions as a component of the mechanism of regulation of food intake. Am. J. Clin. Nutr. 8:547-561.

McCormack, J. G., (1982) The regulation of fatty acid synthesis in brown adipose tissue by insulin. Progr. Lip. Res. 21:195-223.

McNab, B.K., (1983) Energetics, body size, and the limits to endothermy. J. Zool. Lond, 199:1-29.

Morgane, P.J., (1979) Historical and modern concepts of hypothalamic organization and function. In: Handbook of the Hypothalamus, Eds. P.J. Morgane and J. Panksepp, Volume 1°pp 1-64.

Nedergaard, J., and Cannon, B., (1984) Regulation of thermogenin expression and activity in brown adipose tissue. in Thermal Physiology, ed J.R.S. Hales, pp 169-173.

Nedergaard, J., and Cannon, B., (1987) Apparent unmasking of [^{H}]GDP binding in rat brown-fat mitochondria is due to mitochondrial swelling. Eur. J. Biochem. 164:681-686.

Nedergaard, J., and Lindberg, O., (1982) The brown fat cell. Int. Rev. Cytol. 74:187-286.

Nedergaard, J., Raasmaja, A., and Cannon, B., (1984) Parallel increases in amount of (^{3}H)GDP binding and thermogenin antigen in brown-adipose-tissue mitochondria of cafeteria-fed rats. Biochem. Biophysical. Res. Commun. 122(3):1328-1336.

Nicholls, D.G., (1974) Hamster brown adipose tissue mitochondria: the control of respiration and the proteon electrochemical potential by possible physiological effectors of the proton conductance of the inner membrane. Eur. J. Biochem. 49:585-593.

Nicholls, D.G., (1976) Hamster brown adipose tissue mitochondria. Puring nucleotide control of the ion conductance of the inner membrane, the nature of the nucleotide binding site. Eur. J. Biochem. 62:223-228.

Nicholls, D.G., (1977). The effective proton conductance of the inner membrane of mitochondria from brown adipose tissue: dependency on proton electrochemical gradient. Eur. J. Biochem. 77:349-356.

Nicholls, D.G., and Locke, R.M., (1984) Thermogenic mechanisms in brown fat. Physiol. Rev. 64(I):1-64.

Niijima, A., (1986) Effect of glucose and other hexoses on efferent discharges of brown adipose tissue nerves. Am. J., Physiol. 251:R240-R242.

Olney, J.W., (1969) Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 146:719-721.

Olney, J.W., (1971) Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. An electron microscopy study. J. Neuropathol. Exp. Neurol. 30:75-90.

Oomura, Y., Mizuno, Y., Shimizu, N., and Minami, T., (1983) Hierarchial information processing of blood chemistry by chemosensitive elements in peripheral and central nervous system. (Abstract) Soc. Neurosci. Abstr. 9:189.

Oomura, Y., Nakamura, T., Sugimori, M., and Yamada, Y., (1975) Effect of free fatty acid on the rat lateral hypothalamic neurons. Physiol. Behav. 14:483-486.

Oomura, Y., Ohta, M., Ishibashi, S., Kita, H., Okajima, T., and Ono, T., (1978) In: Recent Advances in Obesity Research II, Ed. G. Bray, (Proc. 2nd Int. Congr. Ob.) pp 17-26.

Oomura, Y., and Yoshimatsu, H., (1984) Neural network of glucose monitoring system. J. Auton. Nerv. Syst. 10:359-372.

Pennington, J.A.T., and Church, H.N., (1980) Bowes and Church's food values of portions commonly used. Philadelphia: Lippincott.

Perkins, M.N., Rothwell, N.J., Stock, M.J., and Stone, T.W., (1981) Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. Nature 289:401-402.

Perry, J.H., Liebelt, R.A., (1961) Extra-hypothalamic lesions associated with gold-thioglucose induced obesity. Proc. Soc. Exp. Biol. and Med. 106:55-57.

Petersen, S., (1978) Feeding, Blood glucose and plasma insulin of mice at dusk. Nature 275 (19):647-649.

Powley, T.L., (1977) The ventromedial hypothalamic syndrome, satiety and a cephalic phase hypothesis. Psychol. Rev. 84:89-126.

Powley, T.L., and Opsahl, C.A., (1974) Ventromedial hypothalamic obesity abolished by subdiaphragmatic vagotomy. Am. J. Physiol. 226:25-33.

Powley, T., and Plocher, T.A., (1980) Hypophysectomy blocks the weight gain and obesity produced by goldthioglucose lesions. Behav. Neural. Biol. 28:300-318

Powley, T.L., and Prechtl, J.C., (1986) Gold thioglucose selectively damages dorsal vagal nuclei. Brain Res. 367:192-200.

Raasmaja, A., Mohell, N., and Nedergaard, J., (1984a) Increased α_1-adrenergic receptor density in brown adipose tissue of cold-acclimated rats and hamsters. Eur. J., Pharmacol. 106:489-498.

Raasmaja, A., Mohell, N., and Nedergaard, J., (1984b) Increased α1-adrenergic receptor density in brown adipose tissue of cafeteria-fed rats. Bioscience Reports 4:851-859.

Richard, D., and Trayhurn, P., (1984) Effect of exercise training on the rates of fatty acid synthesis in mice. Can. J. Physiol. Pharmacol. 62:695-699.

Ricquier, D., Mory, G., Bouillaud, F., Thibault, J., and Weissenbach, J., (1984) Rapid increase of mitochondrial

uncoupling protein and its mRNA in stimulated brown adipose tissue. FEBS Letters, 178(2):240-244.

Rothwell, N.J., and Stock, M.J., (1979) A role for brown adipose tissue in diet-induced thermogenesis. Nature (London) 281:31-35.

Rothwell, N.J., and Stock, M.J., (1982a) Neural regulation of thermogenesis. Trends in Nuerological Sciences 5:124-126.

Rothwell, N.J., and Stock, M.J., (1982b) Effect of chronic food restriction on energy balance, thermogenic capacity, and brown-adipose-tissue activity in the rat. Bioscience Rep. 2:543-549.

Rothwell, N.J., and Stock, M.J., (1983a) diet induced thermogenesis. In: Mammalian Thermogenesis, Eds. L. Girardier and M.J. Stock, pp 208-233.

Rothwell, N.J., and Stock, M.J., (1983b) Acute effects of fat and carbohydrate on metabolic rate in normal, cold-acclimated and lean and obese (fa/fa) Zucker rats. Metabolism 32(4):371-376.

Rothwell, N.J., and Stock, M.J., (1984) Tissue blood flow in control and cold-adapted hyperthyroid rats. Can. J. Physiol. Pharmacoll 62:928-933.

Rothwell, N.J., and Stock, M.J., (1985) Biological distribution and significance of brown adipose tissue. Comp. Biochem. Physiol. 82A(4):745-751.

Rothwell, N.J., and Stock, M.J., (1987) Effect of environmental temperature on energy balance and thermogenesis in rats fed normal or low protein diets. J. Nutr. 117:833-837.

Rothwell, N.J., Stock, M.J., and Sudera, D.K., (1986) Thermogenic responses to adrenoceptor agonists and brown fat adrenoceptors in overfed rats. Eur. J. Pharmacol. 125:313-323.

Rothwell, N.J., Stock, M.J., and Warwick, B.P., (1983a) The effect of high fat and high carbohydrate cafeteria diets on diet-induced thermogenesis in the rat. Int. J. Obesity 7:263-270.

Rothwell, N.J., Stock, M.J., Warwick, B.P., and Winter, P.D.O'B. (1983b) Diurnal variations in circulating hormone levels and brown adipose tissue activity in "cafeteria"-fed rats. Comp. Biochem. Physiol. 75A(3):461-465.

Saito, M., and Bray, G., (1983) Diurnal rhythm for corticosterone in obese (ob/ob) diabetes (db/db) and gold-thioglucose-induced obesity in mice. Endocrinol. 113:2181-2185.

Schacterle, G.R., and Pollack, R.L., (1973) A simplified method for the quantitative assay of small amounts of protein in biologic material. Anal. Biochem. 51:654-655.

Schwartz, J.H., Young, J.B., and Landsberg, L., (1983) Effect of dietary fat on sympathetic nervous system activity in the rat. J. Clin Invest. 72:361-370.

Sclafani, A., (1984) Animal models of obesity: classification and characterization. Int. J. Obesity 8:491-508.

Sclafani, A., and Kirchgessner, A.L., The role of the medial hypothalamus in the control of food intake: an update. In: Neural and Humoral Controls of Food Intake, eds. R.C. Ritter, S. Ritter, and C.D. Barnes, Academic Press, In press.

Silva, J.E., and Larsen, P.R., (1983) Adrenergic activation of triiodothyronine production in brown adipose tissue. Nature (London) 305:712-713.

Silva, J.E., and Larsen, P.R., (1985) Potential of brown adipose tissue type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats. J. Clin. Invest. 76:2296-2305.

Silva, J.E., and Larsen, P.R., (1986a) Interrelationships between thyroxine, growth hormone and the sympathetic nervous system in the regulation of 5'-iodothyronine deiodinase in rat brown adipose tissue. J. Clin. Invest. 77:1214-1223.

Silva, J.E., and Larsen, P.R., (1986b) Hormonal regulation of iodothyronine 5'deiodinase in rat brown adipose tissue. Am. J. Physiol. 251:E639-E643.

Silva, J.E., Leonard, J.L., Crantz, F.R., and Larsen, P.R., (1982) Evidence for two tissue-specific pathways for in vivo thyroxine 5'-deiodination in the rat. J. Clin. Invest. 69:1176-1179.

Sinha, Y.N., Salocks, C.B., and Vanderlaan, W.P., (1975) Prolactin and growth hormone secretion in chemically induced and genetically obese mice. Endocrinol. 97:1386-1393.

Slinde, E., Pedersen, J.I., and Flatmark, T., (1975) Sedimentation coefficient and buoyant density of brown adipose tissue mitochondria from guinea pigs. Anal. Biochem. 65:581-585.

Smith, C.J.V., (1983) Treatment of mice with monosodium glutamate does not inhibit a subsequent response to gold thioglucose. Experientia 39: 156-157.

Smith, R., and Horwitz, B., (1969) Brown fat thermogenesis. Physiol. Rev. 49:330-425.

Stellar, (1954) The physiology of motivation. Psychol. Rev. 61:5-22.

Storlien, L.H., (1985) The role of the ventromedial hypothalamic area in periprandial glucoregulation. Life Sciences 36:505-514.

Sullivan, A.C., and Gruen, R.K., (1985) Mechanisms of appetite modulation by drugs. Fed. Proc. 44:139-144.

Sundin, U., Mills, I., and Fain, J.N., (1984) Thyroid-catecholamine interactions in isolated rat brown adipocytes. Mebatolism 33:1028-1033.

Swanson, L.W., and Kuypers, H.G.J.M., (1980) The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labelling methods. J. Comp. Neurol. 194:555-570.

Swanson, L.W., and Sawchenko, P.E., (1983) Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Ann. Rev. Neurosci. 6:269-324.

Swartz, H.A., Christian, J.E., and Andrews, F.N., (1960) Distribution of sulfur-^{35} and gold-^{198}-labelled gold thioglucose in mice. Am. J. Physiol. 199(1):67-70.

Takasaki, Y., (1978) Studies on brain lesion by administration of monosodium L-glutamate to mice. 1. Brain lesions in infant mice caused by administration of monosodium L-glutamate. Toxicology 9: 293-305.

Tanaka, K., Shimada, M., Nakao, K., and Kusunoki, T., (1978) Hypothalamic lesion induced by injection of monosodium glutamate in suckling period and subsequent development of obesity. Exp. Neurol. 62:191-199.

Tokuyama, K., and Himms-Hagen, J., (1986) Brown adipose tissue thermogenesis, torpor, and obesity of glutamate-treated mice., Am. J. Physiol. E407-E415, 1986.

Tokuyama, K., and Himms-Hagen, J., (1987) Increased sensitivity of the genetically obese (ob/ob) mouse to corticosterone. Am. J. Physiol. 252:E202-E208.

Torvik, A., (1956) Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures. J. Comp. Neurol. 106:51-141.

Trayhurn, P., Ashwell, M., Jennings, G., Richard, D., and Stirling, D.M., (1987) Effect of warm or cold exposure on GDP binding and uncoupling protein in rat brown fat. Am. J. Physiol. 252:E237-E243.

Trayhurn, P., and Jennings, G., (1986) Evidence that fasting can induce a selective loss of uncoupling protein from brown adipose tissue mitochondria of mice. Bioscience Reports, 6(9):805-810.

Triandafillou, J., Gwilliam, C., and Himms-Hagen, J., (1982) Role of thyroid hormone in cold-induced changes in rat brown adipose tissue mitochondria. Can. J. Biochem. 60:530-537.

Triandafillou, J., Hellenbrand, W., and Himms-Hagen, J., (1984) Trophic response of hamster brown adipose tissue: roles of norepinephrine and pineal gland. Am. J. Physiol. 247:E793-E799.

Vander Tuig, J.G., Knehans, A.W., and Romsos, D.R., (1982) Reduced sympathetic nervous system activity in rats with ventromedial hypothalamic lesions. Life Sci. 30:913-920.

Vander Tuig, J.G., Ohshima, J.G., Yoshida, T., Romsos, D.R., and Bray, G., (1984) Adrenalectomy increases norepinephrine turnover in brown adipose tissue of obese (ob/ob) mice. Life Sci. 34:1423-1432.

Visser, T.J., Leonard, J.L., Kaplan, M.M., and Larsen, P.R., (1982) Kinetic evidence suggesting two mechanisms for iodothyronine 5'-deiodination in rat cerebral cortex. Proc. Natl. Acad. Sci. 79:5080-5084.

Wade, G.N., (1982) Obesity without overeating in golden hamsters. Physiol. and Behavior 29:701-707.

Webb, G.P., Jagot, S.A., and Jakobsen, M.E., (1982) Fasting-induced torpor in Mus musculus and its implications

for the use of murine models for human obesity studies. Comp. Biochem. Physiol. 72A(1):211-219.

Webb, G.P., Jagot, S.A., Rogers, P.D., and Jakobsen, M.E., (1980) The effects of fasting on thermoregulation in normal and obese mice. IRCS Medical Science: Biochemistry 8:163-164.

Winer, B.J., (1971) Statistical Principles in Experimental Design, Eds. N. Garmezy, R.L. Solomon, L.V. Jones and H.W. Stevenson. McGraw-Hill pp 261-308.

Wu., S.Y., Stern, J.S., Fisher, D.A., and Glick, A., (1987) Cold-induced increase in brown fat thyroxine 5'-monodeiodinase is attenuated in the Zucker obese rat. Am. J. Physiol. 252:E63-67.

York, D.A., Marchington, D., Holt, S.J., and Allars, J., (1985) Regulation of sympathetic activity in lean and obese Zucker (fa/fa) rats. Am. J. Physiol. 249:E299-E305.

Young, J.B., Kaufman, L.N., Saville, M.E., and Landsberg, L., (1985) Increased sympathetic nervous system activity in rats fed a low-protein diet. Am. J. Physiol. R627-R637.

Young, J.B., and Landsberg, L., (1980) Impaired suppression of sympathetic activity during fasting in the gold-thioglucose-treated mouse. J. Clin. Invest 65:1086-1094.

Young, J.B., Saville, E., Rothwell, N.J., Stock, M.J., and Landsberg, L., (1982) Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue of the rat. J. Clin. Invest. 69:1061-1071.

Young, J.K., Nance, D.M., and Gorski, R.A., (1978a) Effects of estrogen upon hypothalamic vulnerability to goldthioglucose in mice. Brain Res. Bull 3(3):231-235.

Young, J.K., Nance, D.M., and Gorski, R.A., (1978b) Dietary effects upon food and water intake and responsiveness to estrogen, 2-deoxyglucose and glucose in female rats. Physiol. Behav. 21:395-403.

Young, J.K., Nance, D.M., and Gorski, R.A., (1979a) Effects of diet upon hypothalamic vulnerability to goldthioglucose in male mice. Brain Res. Bull. 4(1):69-74.

Young, J.K., Nance, D.M., Gorski, R.A., and Gordon, J.H., (1979b) Further characterization of fat diet-induced changes in responsiveness to estrogen and glucose. Brain. Res. Bull. 4:61-68.

Zaror-Behrens, G., and Himms-Hagen, J., (1983) Cold-stimulated sympathetic activity in brown adipose tissue of obese (ob/ob) mice. Am. J. Physiol. 244:E361-E366.

Zaror-Behrens, G., and Himms-Hagen, J., (1984) Turnover of noradrenaline in brown adipose tissue BAT of genetically (ob/ob) and goldthioglucose (GTG) obese mice. Fed. Proc. 43:1081 (Abstract)

Zaror-Behrens, G., and Himms-Hagen, J., Brown adipose tissue of mice with goldthioglucose-induced obesity: noradranaline turnover. Submitted for publication.